CHARACTERIZATION OF A PEA RECOMBINANT INBRED POPULATION FOR RESISTANCE TO HEAT AT FLOWERING

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ABSTRACT

Field pea (*Pisum sativum* L.) as a cool season legume crop is sensitive to high day time temperature, especially during flowering. A population of 107 recombinant inbred lines (RILs) known as PR-11 was made from the cross of CDC Centennial (heat tolerant cultivar) X CDC Sage (heat sensitive cultivar) with the objectives of screening heat tolerant traits during flowering and subsequent seed development, and to map the quantitative trait loci (QTLs) responsible for these traits. Experiments were carried out in 2012-2014. PR-11 was seeded at normal seeding dates in 2012 and 2013 at Saskatoon (52 º12'N, 106º63'W) and Rosthern (52º66'N, 106º33'W) in Canada, and in 2014 PR-11 was seeded at both normal and late seeding (three weeks later than normal) dates at one location, Saskatoon.

Correlation analyses demonstrated that the duration of flowering (DOF) was positively associated with final seed yield under both normal and late seeding date conditions. Yield component traits on the main-stem [reproductive node number (Rnode), pod number (Pod), seed number per pod (Seed), single seed weight (SSW)] were significantly associated with main-stem seed yield, among which pod number appeared to be the component most positively associated with seed yield. However, yield on the main-stem was not significantly associated with seed yield at the plot level, which inferred that the contribution of seed yield on side branches was important.

A genetic map consisting of 369 SNPs markers with a total coverage of 746 cM was developed using JoinMap 4.0. A total of 14 QTLs were detected under environments with normal seeding date, six for flowering traits, and eight for yield component traits. Eight QTLs were identified at late seeding, four for flowering traits and four for yield component traits. The total variation in days to flowering (DTF), DOF, Pod, Seed, SSW and grain yield that were each

explained by the QTLs under normal seeding environments was 24 %, 43%, 15%, 32%, 34% and 21%, respectively. The QTLs together accounted for 43% of DTF variation, 14% of DOF variation, 17% of Pod variation, 12% of SSW variation and 12% of grain yield variation at the late seeding date.

Lines PR-11-2, PR-11-88 and PR-11-91 performed as the top yielding lines under both normal and late seeding environments, and could be considered as heat tolerant lines.

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ABBREVIATIONS

CDC: Crop Development Centre

cM: centi-morgan

CO_2eq: carbon dioxide equivalent

CTD: canopy temperature depression

CV: coefficient of variation

DOF: duration of flowering

DTF: days to flowering

DTFT: days to flowering termination

FAOSTAT: Food and Agriculture Organization of the United Nations Statistics Division

Fv: chlorophyll variable fluorescence

GDD: growing degree day

GHG: greenhouse gas

h^2: broad-sense heritability

ha: hectare

LG: linkage group

LOD: logarithm of odds ratio

LSD: least significant difference

MAS: marker assisted selection

Node: total nodes per plant

Pod: pod number per plant

QTL: quantitative trait loci

RCBD: randomized completely block design

RIL: recombinant inbred line

Rnode: reproductive nodes per plant

RO: reproductive organ

SD: standard deviation

Seed: seed number per pod

SNP: single nucleotide polymorphism

SSW: single seed weight

T: tonnes

σ^2_e: environment variance

σ^2_g: genotype variance

σ^2_{gl}: genotype by location interaction variance

σ^2_{gy}: genotype by year interaction variance

σ^2_p: phenotypic variance

1.0 INTRODUCTION

Dry pea (*Pisum sativum* L.) is an annual herbaceous plant, and is classified as a cool season crop grown in most temperate areas of the world. It originated in western Asia and was introduced to Europe, and then it was spread to other parts of the world. In Canada, pea is mainly grown in the western prairie region as a grain crop. The crop is known as dry pea, field pea, grain pea or pea. By 2015, production area had increased to 1.5 million hectares (ha), more than 30 times that from 1981 (0.05 million ha). Now Canadian production of pea has reached 3.2 million tonnes (T) accounting for 35% of global production (Statistics Canada, 2015). Since 2011, the annual value of exported dry peas from Canada has exceeded $1.0 billion US according to data published by the Food and Agriculture Organization of the United Nations Statistics Division (FAOSTAT, 2012).

Heat stress is one of the major abiotic stresses limiting the world's agricultural production. Lobell and Field (2007) reported the global yield of major crops (wheat, maize and barley) experienced large losses due to elevated temperature, and they estimated that since 1980 the combined loss of these crops was worth $5 billion US per year. As a cool-season legume crop, pea is very susceptible to high temperature stress. Pea production starts to suffer a reduction when the maximum daytime air temperature exceeds 25 ℃ (Guilioni et al, 2003). When air temperature is over 30 ℃ for just a few hours a day, the damage to plants is regarded as moderately severe, and severe when maximum air temperature exceeds 35 ℃ for similar periods (Munier-Jolain and Carrouée, 2010). Various crops such as wheat (Sharma et al, 2005), rice (Weerakoon et al, 2008) and cotton (Singh et al, 2007) have been evaluated for their response to heat stress at reproductive growth stages using agronomic, phenological, morphological and

physiological approaches; similar studies on pea are limited. The average annual temperatures for Canada as a whole has increased by 1.4 ℃ for the period from 1948 to 2009 (Environment Canada, 2010), and the average summer temperature across the nation has also warmed by 1.4 ℃ (Environment Canada, 2014). In a warming climate, the pea crop is going to be stressed more often, resulting in shorter times of growth and potentially substantial reductions in yield and quality.

Therefore, there is an urgent need to study the physiological mechanisms associated with reproductive failure of pea in order to identify resistant genotypes to breed pea that can escape stress or have improved resistance to high temperature stress. In addition to conventional breeding methods, marker-assisted selection (MAS) for heat tolerance has proved to be helpful in wheat (Farooq et al, 2011), cowpea (Lucas et al, 2013) and faba bean (Lavania et al, 2015). Although MAS for heat tolerance has not yet been applied in pea, advances have been made in QTL mapping and subsequent MAS for other abiotic stress resistance such as frost and salinity in pea during the last decade (Bohra et al, 2014). With the development of next generation sequencing technology and a high-density genetic map of pea (Duarte et al, 2014), identification of QTLs linked to heat tolerance traits will become easier.

In this thesis I will focus attention on the influence of high temperature on pea's reproductive growth based on three facts: 1) high summer temperature usually coincides with pea flowering in Saskatchewan; 2) most crops are more sensitive to heat stress during flowering and the following seed development stage as compared to vegetative stages; and 3) heat causes abortion of floral buds and flowers, resulting in appreciable loss in yield.

The hypotheses of this research are as follows:

1. The field pea recombinant inbred lines population PR-11, which was derived from the cross of CDC Centennial (more heat resistant in term of yield) X CDC Sage (relatively heat sensitive), will produce a range of genetic variation regarding the response of flowering and yield related traits to heat stress.

2. These responses are controlled by several regions of the pea genome which can be identified using linked molecular markers and phenotypic variability among the RILs.

The overall objective of this research is to investigate the impact of heat stress on flowering and yield in PR-11, identify potential heat tolerance traits and, thereby, determine the genetic control of these traits.

2.0 LITERATURE REVIEW

2.1 Field pea, value and production

2.1.1 Field pea and its nutritional value

Field pea (*Pisum sativum* L.) is an annual herbaceous plant, and is a cool season crop widely grown in the temperate zones of the world. It belongs to the family of pulse crops, referring to legume crops with edible seeds high in protein and starch, and relatively low in lipid content. Other major pulse crops include common bean, lentil, chickpea and faba bean. Unlike green pea or vegetative pea that is mainly consumed as fresh pods, fresh seed or as a canned vegetable, field pea or dry pea is marketed as dry, shelled grain for both human food and livestock feed.

Field pea is well recognized for its highly nutritional seeds. They are very low in fat, high in complex carbohydrate including soluble and insoluble fiber (Wang and Daun, 2004), and are good sources of protein (twice that of cereals), vitamins, and minerals (Bassett et al, 2010). Research in America has demonstrated that replacing energy-dense foods with peas and other legumes had positive effects in the prevention and management of obesity and related chronic diseases, such as cardiovascular diseases and metabolic syndrome (Rebello et al, 2014). Other studies implied that pea benefitted prevention of diabetes and cancer (Mitchell et al, 2009; Roy et al, 2010) . In addition, field pea is an excellent protein supplement in swine, cow, feeder calf, dairy and poultry rations. In summary, dry pea has an abundant source of nutrition beneficial to both humans and animals.

2.1.2 Production

As one of the earliest cultivated crops, the domestication of field pea can be traced back to 9,000 BC in present-day southern Turkey and northern Syria. Then it was introduced into Europe before it was later spread to North America. The annual growing area and production of pea in the world ranges from six to seven million ha and ten to thirteen million tonnes, respectively, which makes it the fourth most important pulse crop after dry bean, chickpea and cowpea in terms of area and third in terms of production after dry bean and chickpea, globally (FAOSTAT, 2014). The top five pea producing countries are Canada, Russia, China, France and India; production figures are based on the average of ten years production from 2003 to 2014 using published data from the FAO STAT database (FAOSTAT, 2014).

The tradition of Canadian farmers growing field pea started 100 years ago. In the beginning, it was only grown in a limited area, but production began to increase and has been growing consistently since World War II. According to the most recent year (2014) of data published by FAO STAT, the seeded area of field pea jumped from 59,500 ha in 1981 to 1.3 million ha (11.3% annual growth rate), its production increased from 110,500 tonnes to 3.8 million tonnes (12.3% annual growth rate), as of 2013. Canada is now the top field pea producing and exporting country in the world, with 3.8 million T of production in 2014 which accounted for 34.5% of global production, of which 2.8 million T were exported (AAFC, 2015). The vast majority of pea is produced in the three prairie provinces (Saskatchewan, Alberta and Manitoba), among which Saskatchewan is the heart of field pea production. In 2014, Saskatchewan produced 60% of Canada's dry pea, making up 55% of pea's global exports which brought $1.2 billion of revenue (Saskatchewan Ministry of Agriculture, 2015).

2.2 Heat stress

2.2.1 Climate change and warming of Canada

Climate change is a global problem causing widespread effects on human and natural systems. Global warming is one of the best-known gradual changes caused by climate change. It also increases the frequency of extreme weather events such as thunderstorms, tornadoes and tropical cyclones. Starting from the late 19th century, both the annual air temperature at the earth and ocean surface, on a global scale, have been consistently rising, with a 1.6 ℃ increase at the land surface and a 0.6 ℃ increase at the ocean surface, respectively (IPCC, 2014). In the previous 100 years, each of the three decades has become progressively hotter than the past three decades. The period from 1983 to 2012 appeared to be the hottest 30-year period within the most recent 1400 years in the northern hemisphere. The causes of the whole climate system's warming are accepted as anthropogenic (caused by humans). Since 1750, anthropologic causes have played the major role by overburning fossil fuels and overloading the atmosphere with greenhouse gases (GHGs) such as carbon dioxide, methane, water vapor, nitrous oxide and sulphur hexafluoride. When sunlight reaches Earth's surface, only a small amount of it is absorbed to warm the earth, and most of the rest is radiated back to the atmosphere in the form of a longer wavelength than incoming sunlight wavelengths. GHGs can absorb some of these wavelengths before they are lost in the space, and as a result they cause further warming of the near atmosphere.

Canada accounts for only 0.5% of the world's population but contributes about 2% of the total global GHGs emissions (IPCC, 2014). Although Canadian per capita greenhouse gas emissions have dropped by 6% (from 22.1 to 20.7 T of GHGs per capita) during 1990 to 2013, Canadians are still among the highest per capita emitters in the world. Over the same period, the

total GHG emissions in Canada rose by 18% from 613 Megatonnes CO_2 eq (CO_2 eq is the measure to demonstrate the greenhouse gas emissions based on one unit global warming potential of CO_2) in 1990 to 726 Megatonnes CO_2 eq in 2013 (Environment Canada, 2014), mainly due to the increase in emissions from energy and agriculture sectors. This increase in the GHG emissions leads to the rise in the national average temperature. From 1948 to 2013, the annual temperature across the country warmed by 1.6 ℃ (Environment Canada, 2013).

In the context of the growing season summer temperature in Canada, the national average temperature for the past 2014 summer was 1.0 ℃ above the baseline average (defined as the mean over the 1961–1990 reference period), which makes it the sixth warmest summer since recording from 1948. The recorded warmest summer was 2012 when the average temperature of the nation was 1.8 ℃ above the baseline average, and the summer of 2012 may have been surpassed by the summer of 2015. Overall, over the period of 1948-2014, the national summer temperature had warmed by 1.4 ℃ (Environment Canada, 2014). As there is no sign of possible significant reductions of GHG emissions in the near future, no doubt both the summer and the yearly average temperature will continue to increase.

2.2.2 Crop yield reduction due to elevated temperature

Therefore, climate change will lead to elevated temperatures because of increased amounts of GHG emissions by humans. The higher temperature would shorten a crop plant's life cycle, cause detrimental damages to the plant's reproductive organs, and thus lower crop production. Besides, persistent high temperatures also reduce the moisture content of soil, which causes drought stress. High temperature stress has become a leading abiotic constraint threatening global agricultural production. Lobell and Field (2007) reported an 8.3 percent yield reduction in the world's number one crop, maize, in response to every 1 ℃ rise in temperature for the period

1961-2002. Similarly in wheat, every 1 ℃ above an optimal temperature could shorten the flowering and grain-filling duration about 5% respectively, thus reducing grain yield accordingly (Lawlor and Mitchell, 2000). In chickpea, increasing the seasonal temperature by 1 ℃ caused a 53 kg ha^{-1} yield loss (Chanders et al, 2008). Information on yield reduction related to elevated temperature was also reported in field pea. Ridge and Pye (1985) stated that the production of field pea grown in the Mediterranean region would decrease by 0.6 T ha^{-1} as a response to every 1 ℃ increase of average temperature during flowering.

2.2.3 Heat stress and the plant threshold temperature

Heat stress can refer to the rise in temperature beyond the upper-temperature threshold for a period, and it induces irreversible detriment to a plant's growth and development. Heat stress can be categorized as chronic or acute based on the timing and duration (Devasirvatham et al, 2012). Chronic heat stress means a relatively long time range of mild stress (the temperature would be a few degree above optimal). Acute stress means a shorter period of extreme heat stress, where the temperature increase may be greater than a few degrees. The prevalence and severity of the two stresses vary from region to region. In the spring-sown field pea areas of western Canada, acute heat stress would cause more damage than chronic heat stress.

Each species has three cardinal temperatures used to describe growth and development in plants: base temperature (also referred as the lower temperature threshold), optimum temperature, and the upper threshold temperature. These cardinal temperatures vary among plant species. For each species, an optimum range of temperatures are seen at different stages of growth. For instance, the best temperatures for vegetative development of wheat (20-30 ℃) and rice (33 ℃) are not the same as those for the reproductive yield phase, which are 15 ℃ for wheat and 23-26 ℃ for rice (Hatfield and Boote, 2008).

The ideal temperature range of cool-season pulses is 10-30 ℃. In general, a daily maximum temperature above 25 ℃ is regarded as the threshold level for heat stress in these crops. Among these legumes, chickpea appears to have the best heat tolerance, followed by faba bean, lentil and field pea, the reverse being true for cold tolerance (Siddique, 1999). For example, chickpea shows its best growth performance when air temperature is around 20-29 ℃ at daytime (Soltani et al, 2006), common beans grow best when temperature is between 16-26 ℃ (Hardman et al, 1990), the best temperature for lentil's cultivation ranges from 15-27 ℃, and the best-growing temperature range for pea is between 13 to 23 ℃ (Mahoney, 1991).

2.2.4 Effect of heat stress on vegetative organs

The optimum temperature for vegetative growth of pea is 15–20 ℃ (Mahoney, 1991). When air temperature is above the optimum, damage to growth and development of vegetative organs begins. In a study of leaf response to high temperature in the greenhouse, Munier-Jolain et al (2010) found that high temperature could reduce the size of all the leaves growing or expanding at the time of the stress; the more severe the stress, the greater the reduction observed. Also, a brief exposure to high temperature (30/25 ℃) could induce early senescence of lower leaves of pea. This senescence was irreversible, and even after plants were returned to the normal 20/15 ℃, damage to these leaves was permanent. Not only did high temperature cause damage to leaf growth, it also negatively affected leaf physiological functions. McDonald and Paulsen (1997) reported that high temperature decreased chlorophyll variable fluorescence (Fv), a measure of injury to photosynthesis, in five pea cultivars. Later when they studied the effect of high temperature on thylakoid activity in the pea cultivar "Alaska", they found the whole-chain photosynthetic activity in thylakoids declined rapidly after heating at 40 ℃ for only 2.5 min. Likewise Kaushal et al (2013) concluded that heat stress drastically reduced stomatal

conductance, leaf water content, chlorophyll, membrane integrity and photochemical efficiency, particularly in heat-sensitive genotypes.

2.2.5 Effect of heat stress on reproductive organs

Depending on the intensity and duration, high temperature affects the reproductive organs (ROs) in different ways. Several studies on mild thermal stress in pea have been published. Guilioni et al (1997) reported that mild stress did not lead to an immediate abscission of reproductive organs, but did result in a delayed abortion of ROs carried by the upper nodes. Under moderate stress, abortion frequency followed a consistent pattern along the stem. The lower down the phytomers (nodes) carrying the pods were, the less abortion was seen. They explained the reason for the pattern of abortion as the ROs located in the distal part of the plant experienced more heat and radiation, and aborted more compared to ROs in the proximal part, although they did not have canopy and node temperature measurements. In a following paper, Guilioni et al (2003) found that mild stress reduced seed number in pea by decreasing the plant growth rate during the critical period for seed set, starting from the beginning of flowering to the beginning of seed filling for the last seed-bearing nodes. They inferred that mild heat stress accelerated the normal termination of node production during the plant's life cycle, i.e., that heat caused early maturation by stopping additional node production.

Compared to the mild high temperature stress, a short period of extreme high temperature at anthesis can cause more detrimental and direct damage to yield. The yield loss is mainly caused by the abscission of ROs, especially flower buds, open flowers and young pods. Studies on the physiological mechanisms of RO abscission have been carried out in various legume crops. During the pre-anthesis stage, reduced pollen viability and pollen production per flower are two of the major causes for RO abscission. In chickpea, 60 min after germinating pollen

grains, the *in vitro* pollen incubation rate was higher (61%) at 25 ℃ compared with 45 ℃ (33%; Jaiwal and Mehta, 1983). Pollen abnormalities have been observed in cowpea at 33/30 ℃ when plants were exposed to heat three days before anthesis (Ahmed et al, 1992), and in common bean nine days after heat treatment (32/27 ℃) prior to anthesis (Porch and Jahn, 2001). Pollen production was reduced 30–50% at 38/30 ℃ compared with 30/22 ℃ in soybean (Koti et al, 2005). High temperature effects on post-anthesis were related to poor pollen germination, poor pollen tube growth on the stigma, and even a subsequent failure of pollen fertilization of the ovule. A negative relationship was determined in pea maintained at high temperature and the number of flowers bearing viable pollen, pollen germination and pollen tube length in a small study (Petkova et al, 2009). This relationship was confirmed in a recent growth chamber study, where Jiang et al (2015) found that when pea plants at anthesis were exposed to 36/18 ℃ day/night for 7 days in a growth chamber, the percentage pollen germination, pollen tube length, pod length, seed number per pod, and the seed–ovule ratio dropped dramatically compared to pea exposed to normal conditions of 24/18 ℃. No visible morphological differences in pollen grains or the pollen surface were observed in two pea cultivars in either the stressed or the controlled environment. Similar information has been reported in chickpea (Devasirvatham et al, 2012) and soybean (Djanaguiraman et al, 2013).

2.3 Adaptation mechanisms to heat stress

The adaptive mechanisms dealing with heat stress can be categorized in an ecological framework like water stress (Arnon, 1992; Bueckert and Clarke, 2013). They can be classified into the following three groups.

(1). Escape mechanisms: these basically involve traits relating to earliness. As heat stress can accelerate phenology, peas that flower early can escape most of seasonal heat stress and

mature earlier by avoiding the terminal stress at the end of the growing season. In breeding programs that have released cultivars adapted to heat and drought, early flowering and maturity have proven to be good heat escape mechanisms and serve as useful criteria for selection for heat resistant cultivars (Hall, 2004). However, research demonstrates a drawback with this escape mechanism which shortens vegetative periods, and reduces yield potential in a year with mild or no stress.

(2). Avoidance mechanisms: canopy temperature depression, leaf reflectance and stomatal opening (transpirational cooling) are important physiological components of heat avoidance. Leaves play a critical role in changing their orientation, morphology, transpiration rate and reflectance (Wery et al, 1993). Also, indeterminate growth habits with a prolonged duration of flowering can be another possible way, because cultivars can recover from a short severe stress and resume flowering. These mechanism work by allowing the plant to maintain a cool canopy, or a reasonable balance of yield, and are best used in moderate but not highly stressful environments.

(3). Tolerance mechanisms: heat tolerance is usually associated with a combination of physiological traits such as cellular membrane thermo-stability, alteration of membrane lipid composition, accumulation of heat shock proteins and specific solutes (proline and glycine; Wahid et al, 2007). In addition, the ‘stay-green’ trait (maintenance of leaf chlorophyll and photosynthetic capacity) is also considered an important factor for heat tolerance in some breeding programs (Fokar et al, 1998). However, these tolerance mechanisms are expensive metabolically, and are found in lower productivity situations like survival, and they can cause reduced yield.

2.4 Breeding strategies for improved heat tolerance

Development and selection of crop varieties are usually aimed at improving yield under existing climatic conditions in a specific target region. With changing climate, in particular episodes of high temperature during the reproductive phase, genotypes with physiological, morphological, and molecular traits unique to heat tolerance are required (Semenov and Halford, 2009). Visual selection, selection for physiological traits linked to plant response to high temperature, empirical selection for yield and more recently marker-assisted selection (MAS), are the four principal selection methods used to improve heat tolerance through breeding (Howarth, 2005).

2.4.1 Conventional breeding methods

Traditional methods for breeding heat resistance are centered on the goal of developing and selecting advanced lines that have greater yields than current cultivars in a hot target production environment, which provides a direct measure of heat resistance. However, screening for heat tolerance in the field is usually difficult due to the lack of suitable screening environments and the lack of knowledge for heat tolerance selection.

When selection occurs under field conditions, two factors make selection difficult. The first is uncontrollable environmental factors (i.e. heat stress is usually accompanied by drought and other abiotic stresses) that reduce the precision and repeatability of such trials. This problem is then compensated for by improved selection methodology; for example, by increasing the number of selection sites and using the mean of all locations for selection, or by using controlled growth chambers. The second factor is that high-temperature conditions in the field are inconsistent from year to year, and cannot be guaranteed. Some breeders have used sophisticated techniques such as field-based heat chambers, which are costly (Cottee et al, 2010). Another

common and more cost efficient method is using late planting or seeding of spring/ summer habit material to induce high levels of heat stress during anthesis and the grain-filling period (Krishnamurthy et al, 2011).

In breeding for heat-stress tolerance, productivity is usually the first priority for selection. Using production components that are associated with yield or high heritability values can result in greater gains. Selection indexes are an alternative in addition to direct selection and correlated response (DeSouza et al, 2012). Besides, as stated in the 'adaptation mechanisms to heat stress' section, many physiological traits related to heat avoidance and tolerance such as canopy temperature depression (CTD), stay green and cellular membrane thermo-stability, may be helpful in indirect selection. However physiological methods are not always viable when large populations need to be assessed- a fairly common situation in most breeding programs. In addition, a deeper understanding of the association between these physiological traits and final yield potential is also required, but such associations are often lacking. Such information has already been reported in wheat (Farooq et al, 2011), whereas there is a large gap in research for field pea. From published literature, CTD appears to be a good indictor because of its easy measurement using an infra-red thermometer, and its association with yield in crops that can maintain partial stomatal opening in stress. Stay-green may be another useful trait (it can be easily and directly selected visually, and with SPAD meters and NDVI) when it has a demonstrated association with yield (Borell et al, 2000).

2.4.2 Marker assisted selection (MAS)

Reported traits linked to yield and heat tolerance are multigenic traits controlled by several genes and different genetic mechanisms (Wahid et al, 2007). Many heat-tolerant traits in the literature are physiological traits, and assessment of them is costly and time consuming. QTL

(quantitative trait loci) mapping and subsequent marker-assisted selection appears to be a promising complement to the conventional breeding approach because MAS allows assessment of numbers, locations, and the magnitude of phenotypic effects and pattern of gene action (Vinh and Paterson, 2005). Also, MAS allows assessment of a great number of traits of interest, expressed at different developmental stages, at one time.

Advances in QTL mapping of heat tolerance have been made in major crops like wheat, rice and maize. Recently in wheat, three QTLs for stay green, which are located on chromosome 1AS, 3BS and 7DS, were identified by Kumar et al (2010). Likewise one QTL for CTD under heat stress was located on chromosome 4A-a (Pinto et al, 2010). Also, Mason et al (2010) identified five stable QTLs for heat susceptibility index of yield components by using a recombinant inbred line population. In rice, mapping QTLs for heat tolerance at the flowering stage have been reported. Ye et al (2012) found two major QTLs which accounted 35% of the variation of spikelet fertility under high temperature. Genetic analysis of spikelet fertility was conducted and 2 and 8 QTLs under optimal and high temperature environment were identified, respectively (Jagadish et al, 2010). In maize, several QTLs for cellular membrane stability, high temperature to pollen germination and pollen tube growth were found (Ottaviano and Gorla, 1991; Frova and Sari-Gorla, 1994).

Mapping QTLs for heat tolerant traits in legumes has lagged behind the major commodity crops mainly due to lack of research both focused on heat stress and funding dedicated to special or minor crops. To date the only known detailed research has been carried out in cowpea (Lucas et al, 2013). In this study, they developed a RIL population of 141 individuals and identified 5 QTLs for pod set per peduncle at high temperature by the use of SNP markers which explained 70% of the phenotypic variation. No study on QTL of heat tolerance has been conducted in pea

at this date (2015), partly due to a delay in research focus on heat stress, the lack of accepted criteria for heat tolerant trait selection, and mostly due to a lag in research investment in QTL mapping in pea. However during the last decade, advances in mapping for biotic stress (*Mycosphaerella* blight, *Ascochyta* blight) and abiotic stress (drought, salinity, frost and lodging) resistance have been made. The summary of previous work in the abiotic stress resistance in pea is listed in Table 2.1. Iglesias-Garcia et al (2015) discovered eight QTLs for relative water content in leaves under water stressful environments, with most of the QTLs locating at the linkage group (LG) III. Consistent QTLs responsible for frost damage evaluated at different locations were identified at LG III, V and VI (Lejeune-Hénaut et al, 2008; Dumont et al, 2009; Klein et al, 2014), and these three researches reported a QTL locating at very similar regions (around 30 cM) at LG III. And for salt resistance, Leonforte et al (2013) detected two QTLs, each at LG III and VII. Tar'an et al (2003) found two QTLs (each at LG III and VI) for lodging resistance which explained a total 58% of its phenotypic variation.

With the increasing attention to the development of high density genetic maps of pea, especially the first high-density pea SNP map defining all seven linkage groups recently (Sindhu et al, 2014), identification of QTLs for heat resistance and subsequent marker-assisted selection is promising and possible. The aim of my thesis is to add QTLs related to heat tolerance to the map of PR-11 developed by Sindhu et al (2014).

Table 2.1 Summary of mapping QTLs for abiotic stress resistance in pea.

Biotic/Abiotic stress Resistance	Name of the population	Marker	Reference
Drought	P665 X Messire	SNP	Iglesias-Garcia et al (2015)
Frost	Champagne X Terese	SNP	Lejeune-Hénaut et al (2008)
	Champagne X Terese	AFLP	Dumont et al (2009)
	China (JI1491) X Cameor	SNP/ SSR	Klein et al (2014)
Salinity	Kaspa X Parafield	SNP	Leonforte et al (2013)
Lodging	Carneval X MP1401	AFLP/ RAPD	Tar'an et al (2003)
	CDC Striker X CDC Carrera	SSR	Liu et al (2011)

3.0 MATERIALS AND METHODS

3.1 Plant materials

PR-11 was derived from a cross between CDC Sage X CDC Centennial (crossing number 3970) made in 2008 at the Crop Development Centre, University of Saskatchewan. CDC Centennial was developed by the Crop Development Centre (CDC), University of Saskatchewan, having characteristics of white flowers, seed with yellow cotyledons, a relatively large seed weight and high yield (Warkentin et al, 2007). CDC Sage was also developed by the Crop Development Centre (CDC) in 2005, with the characteristics of white flowers and green cotyledons (Warkentin et al, 2006). The RIL population was developed with the objective of mapping genes related to heat tolerance, with CDC Centennial having greater heat tolerance than CDC Sage based on indirect evidence in yield trials conducted in 2011(Fig 3.1). Also the population was expected to segregate for flowering time, cotyledon color, lodging resistance, seed weight, and seed protein concentration.

The RIL population was derived from a single F_1 plant. Generations were advanced to F_7 by single seed descent in the Agriculture Greenhouse, University of Saskatchewan. Originally, 113 recombinant lines were developed. From 2013, only 107 lines were left; line 45, 47, 107, 111, 112 and 113 were missing or seed was absent. In order to avoid unbalanced data, the common 107 lines were used in data analysis of field performance.

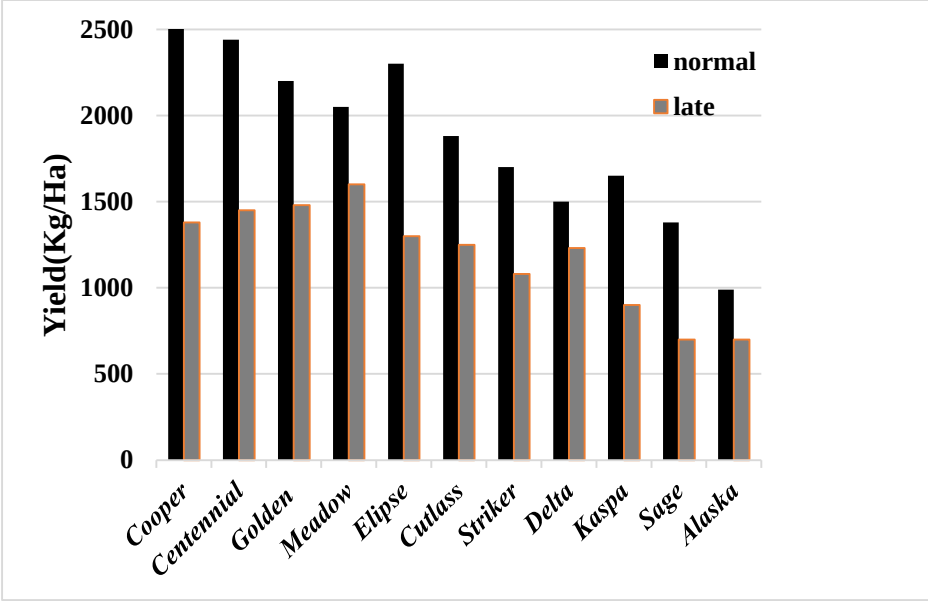


Figure 3.1 Average yields over three locations (Milden, Outlook and Saskatoon) of 11 tested cultivars at two seeding dates in 2011. The cultivars with abbreviated names are CDC Centennial, CDC Golden, CDC Meadow, CDC Striker, and CDC Sage. Unpublished data from Crop Physiology group (University of Saskatchewan).

3.2 Field trials

3.2.1 Experimental design

The experiment was conducted in three years (2012, 2013 and 2014). In 2012 and 2013, the field trials were conducted at Saskatoon (52º12'N, 106º63'W) and Rosthern (52º66'N, 106º33'W) in Saskatchewan, Canada. Within each location, a randomized completely block design (RCBD) with two blocks was used. In 2014, the field trial was conducted only at Saskatoon but with two seeding dates; normal and late (three weeks later). At each seeding date, a RCBD experimental design with three blocks was used. The late seeding date would be expected to generate heat stress on the crop by delaying flowering into mid-July and early August, where maximum daytime temperature were likely to be greatest.

Every year each RIL was planted in 1m ×1m micro-plots with four rows.

Seeding date details were as follows:

2012 PR-11	Saskatoon-May 16, Rosthern- May 11	F8 lines.
2013 PR-11	Saskatoon-May 13, Rosthern- May 1	F9 lines.
2014 PR-11	Saskatoon Normal-May 14, Late-June 4	F10 lines.

3.2.2 Management of field trials

Field management was mainly focused on the control of broadleaf weeds. Management was similar each year. After harvest, a 11.3 rate of Edge (ethalfluralin) and 1/3 rate of Pursuit (imazethspyr) were applied for controlling weeds in the next growing season. Then a full rate of the herbicide product Roundup (glyphosate) was sprayed before seeding, and when pea plants grew to 3-6 nodes, a full rate of the herbicide Viper (imazamox + bentazon) was applied in the field. At the 9-node stage, full rates of Centurion (clethodim) and Axial (pinoxaden) were sprayed. Then Basagran (bentazon) was applied just before peas started to flower. When peas matured, Reglone (diquat) was applied to dry the plants in order to harvest easily. The scientific names, formulations and rates of these products are listed in Appendix A.

3.3 Phenotyping

Flowering related traits days to flowering (DTF), days to flowering termination (DTFT) and duration of flowering (DOF) were recorded and yield component traits were measured after harvest. These components were number of reproductive nodes on the main stem (Rnode), pod number on the main stem (Pod), seed number per pod (Seed) and single seed weight (SSW). Each year when plants started to flower, every two days plots were measured for flowering traits. DTF was defined as 50% of plants within the micro-plot having an open flower. DTFT was defined as 50 % of plants within the micro-plot having reached flower termination. Duration of flowering was calculated as DTFT minus DTF (Maurer et al, 1966).

When RILs reached maturity at the end of August, two representative plants for each RIL micro-plot were hand harvested for yield component traits (Rnode, Pod, Seed and SSW) from the main stem only in the physiology lab, at Department of Plant Science, University of Saskatchewan. The average data from these two subsampled plants were used. In addition, the grain yield of each RIL micro-plot was measured after combine harvest.

In 2012, all the phenotypic traits were measured by Janet Pritchard and Mohammed Tahir at Crop Physiology, University of Saskatchewan. In 2013, Donna Lindsay, Liping Liu and Rosalind Bueckert took notes of flowering traits, and I measured all the yield component traits. In 2014, I measured all the flowering and yield related traits.

3.4 Genotyping

Genotyping was conducted by the Pulse Molecular Breeding group, University of Saskatchewan. DNA was extracted using a modified CTAB method described by Miesel et al (2005). The 106 lines (the sample from line 83 was absent and was not genotyped) and two replications of the parents were screened against a panel of 1536 GoldenGate markers. Information of the GoldenGate assay (Ps1536 OPA) and a standard GoldenGate protocol for genotyping is reported in Sindhu et al (2014).

3.5 Statistical analysis

3.5.1 Phenotypic data analysis

In order to avoid unbalanced data, for phenotypic data in 2014, I used the mean of the value of Block 2 and 3 for each specific trait as the final data for block 2. In this manner, the experiment became a RCBD with 2 blocks at each location-year. The Proc Means procedure of SAS software (SAS Institute Inc. 2015 Version 9.4) was used to display the minimum,

maximum, mean, standard deviation, and standard error of mean of each trait at each individual location-year.

The 2012 growing season was much wetter than average (Section 4.1, Table 4.1), and the RILs at Saskatoon experienced flooded soil conditions and data collected showed a large variance compared to the other location-years. So 2012 Saskatoon data were omitted from the following statistical analyses. As a result, my data became unbalanced. In order to study the effects of environmental factors (location and year) and genotypes in the traits measured, the statistical analysis of phenotypic data was divided into three aspects: 1) analysis of variance for RILs evaluated in 2012 and 2013 at Rosthern, 2) analysis of variance for RILs in 2013 at Saskatoon and Rosthern, 3) analysis of variance for RILs grown in 2013 and 2014 at Saskatoon.

Table 3.1 Summary of the location-year information within each of the three aspects of analysis of variance and the following heritability calculation.

Aspect	Year	Location
1	2012, 2013	Rosthern
2	2013	Rosthern, Saskatoon
3	2013, 2014	Saskatoon

For aspects 1 and 3, the SAS Proc Mixed procedure was used with year, genotype and the interaction of year and genotype as fixed factors, and where block was nested within year as a random factor. For aspect 2, a similar SAS Proc Mixed procedure was used with location, genotype and the effect of genotype-by-location interaction as fixed factors, whereas block nested within location was considered a random factor. In addition, the variance of RILs grown at two different seeding dates in 2014 was analyzed for homogeneity. Two different seeding dates, genotypes and their interactions were considered as fixed effects, whereas blocks nested within seeding date was considered random effects for the estimation of means for each RIL.

Similarly, the analysis of heritability was divided into the same three aspects as in the analysis of variance section. For aspects 1 and 3, the SAS Proc Mixed procedure was used, and genotype, years, their interactions and block nested within years were considered random factors for the estimation of variance components. The phenotypic variance was estimated as $\sigma_P^2 = \sigma_G^2 + (\sigma_{G\times Y}^2 / y) + (\sigma_e^2/ry)$, where σ_G^2 was the estimated genotypic variance, $\sigma_{G\times Y}^2$ was the genotype year interaction, y was the number of years tested, and r was the number of blocks per year. For aspect 2, a similar SAS Proc Mixed procedure was used in which genotype, location, their interactions and block nested within year were considered random factors. The phenotypic variance was estimated as $\sigma_P^2 = \sigma_G^2 + (\sigma_{G\times L}^2 / l) + (\sigma_e^2/rl)$, where σ_G^2 was the estimated genotypic variance, $\sigma_{G\times L}^2$ was the genotype location interaction, y was the number of locations tested, r was the number of blocks per location. Broad-sense heritability for each trait was defined as $\mathrm{H}^2 = \sigma_G^2/\sigma_P^2$ (Singh et al, 1993).

The greater than average amount of rainfall and disease development in 2012 at Rosthern resulted in the measured traits having greater variation compared to data from other years (the average of each of the measured traits among RILs was either high or low compared to that of the RILs population at other location-years), thus data were divided into three sub-datasets (Table 3.2), namely the 2012 Normal seeding date, the 2013/14 Normal, and the 2014 Late seeding date. The frequency distribution of the RILs population for each traits based on each of these three datasets are shown in the chapter “Appendices”.

Table 3.2 Summary of the three datasets used for the frequency distribution figures with the details of the component data of each dataset.

Dataset name	Year	Location	Seeding date
2012 Normal	2012	Rosthern	Normal
2013/14 Normal	2013	Rosthern, Saskatoon	Normal
	2014	Saskatoon	Normal
2014 Late	2014	Saskatoon	Late

The weather conditions of 2013 and 2014 growing seasons were similar to the ten year average, thus the data from the 2013/14 Normal dataset was considered representative of the RILs under the normal seeding environment. Therefore, I only used the results of the association analysis based on the RILs evaluated from the 2013/14 Normal dataset for the results of RILs at normal seeding, and placed them in the corresponding section in chapter 4.0 (section 4.5.1). The results based on RILs evaluated from the 2012 Normal dataset were placed in the appendix. In addition, the dataset 2014 Late was used to represent RILs at late seeding (section 4.5.2). Associations among the nine measured traits at normal and late seeding were each analyzed through the SAS Proc Corr procedure.

In order to discover the relative contribution of four yield component traits (Rnode, Pod, Seed and SSW) to the main-stem seed yield at normal and late seeding, I used a path coefficient analysis using the SAS Proc Calis procedure. The path analysis method was first suggested by Wright (1921). Similar to the association analyses above, the average of each RIL evaluated at 2013 and 2014 normal seeding plots (2013/14 dataset) was used to represent the final RIL value under the normal seeding environment. Likewise, the mean of each RIL over two blocks at the 2014 late seeding (2014 Late dataset) was used to represent the final RIL value at late seeding.

3.5.2 Linkage map construction and QTL analysis

SNP (Single nucleotide polymorphism) markers linked to linkage groups (LGs) were determined at a minimum LOD (logarithm of odds ratio) value of 5 using JoinMap 4.0 (Van Ooijen, 2009). Then the map order of each linkage group was finalized with the use of regression mapping. The recombination frequencies were converted into centiMorgan (cM) through the Kosambi mapping function. A graphical map was generated by MapChart 2.2 (Voorrips, 2002).

The detection of QTLs associated with the traits was based on the 2013/14 Normal dataset and the 2014 Late dataset, respectively. The 2013/14 Normal dataset was used to represent the RILs population at normal seeding and the 2014 Late was used for the RILs population under one late seeding environment. In addition, the results of QTL identification based on the 2012 Normal dataset was given in the chapter appendix. For each dataset, the identification of QTLs was divided into three steps. Firstly, a preliminary detection of putative QTLs with a LOD value around or over 3 was conducted by an interval mapping method (a single-QTL model) using MapQTL® 6 (Van Ooijen, 2009). Secondly, 1000 permutations were tested in order to determine the threshold of LOD at which QTLs were significantly correlated with the trait. Finally, markers with the highest LOD score (the highest score must exceed the significant threshold LOD score) in the different linkage groups were selected as cofactors, and a multiple QTL method was rerun to identify the true QTLs for the traits of interest.

4.0 RESULTS

4.1 Growing season weather information

The average monthly temperature of the growing seasons (May to August) and the total precipitation (May to August) of each location-year are given in Table 4.1. The mean monthly temperature were similar across three years at both locations, although the 2012 growing season was much wetter than the 2013 and 2014 growing seasons. Plots at Saskatoon 2012 experienced significant rain and saturated soil (flood), data collected at this location were highly variable compared to the other location-years, and plots at Rosthern 2012 also experienced flooding to a lesser extent.

Table 4.1 May to August average monthly temperature and total precipitation of the growing season at Saskatoon and Rosthern in 2012, 2013 and 2014.

Location	Soil Zone	Mean temperature (℃)	Total precipitation (mm)
2012 Saskatoon (Omitted)	Dark Brown	15.7a	380a
2012 Rosthern	Black	15.9b	410c
2013 Saskatoon	Dark Brown	16.1a	146a
2013 Rosthern	Black	15.9b	200c
2014 Saskatoon	Dark Brown	15.1a	256a

Note: a. Based on data from Environment Canada; **b**. based on the average of Saskatoon and Prince Albert; **c**. based on data from Saskatchewan Ministry of Agriculture yearly final crop reports.

Based on the average days to flowering (DTF) and days to flowering termination (DTFT) of the RILs at each location-year, the corresponding mean of the daily maximum temperature and daily average temperature was calculated at each individual location-year, the number of days with the maximum temperature over 27 ℃ was also counted at each location-year (Table

4.2). RILs at Rosthern in 2012 received the most heat stress, followed by 2014 late-seeding at Saskatoon, 2013 Saskatoon, 2014 normal-seeding at Saskatoon, and 2013 Rosthern. Correlation of the daily average and daily maximum temperature with the duration of flowering and the final grain yield were analyzed in the Correlation section (refer to section 4.5.3, Table 4.12).

Table 4.2 Summary of average daily maximum temperature, mean of daily temperature and the frequency of daily maximum temperature over 27 ℃ during the flowering stage at each location.

Location-year	Mean of daily maximum temperature (℃)	Mean of daily temperature (℃)	Frequency of high temperature
2012 Rosthern	26.0	20.4	9
2013 Saskatoon	24.4	18.3	3
2013 Rosthern	22.8	16.8	1
2014 Saskatoon (normal seeding)	23.8	17.7	2
2014 Saskatoon (late seeding)	25.5	19.3	4

Based on the average of DTF of RILs at each location-year, the corresponding average of daily temperature from sowing to flowering and growing-degree days (GDDs) within the same period are provided in Table 4.3. No significant correlations were found among these three factors.

Table 4.3 Summary of the means of daily temperature from sowing to flowering and growing-degree days (GDD) based on the average days to flowering (DTF) of RILs at each location.

Location-year	Average daily Temperature (℃)	GDD	Mean of DTF (day)
2012 Rosthern	14.4	559.6	58
2013 Saskatoon	15.6	529.7	50
2013 Rosthern	16.0	596.6	53
2014 Saskatoon (normal seeding)	14.7	543.8	56
2014 Saskatoon (late seeding)	15.8	525.4	47

Note: base temperature used in the GDD calculation was 5 ℃.

4.2 Phenotypic data summary

Table 4.4 provides the mean of RILs for all nine traits at each site with the coefficient of variation (CV). Although for each specific trait the means were different among location-years, the CVs were similar which implied no significant difference in the variability of data at different location-years. This confirmed the repeatability and precision of the experiment. Days to flowering (DTF), days to flowering termination (DTFT) and the duration of flowering (DOF) in the late-seeding date plots 2014 were lower than these from the normal-seeding date, showing late-seeding induced earliness in RILs, RILs flowered early and also finished flowering early. In addition, single seed weight and yield at the late-seeding plots were lower than those at the normal seeding date, whereas no differences were seen in the traits of total node number per plant (Node), reproductive node number per plant (Rnode) and pod number per plant (Pod).

Table 4.4 Mean and Coefficient of variation of nine traits for 107 RILs of CDC Centennial X CDC Sage population in individual year at individual location.

Trait		2012 Rosthern	2013 Saskatoon	2013 Rosthern	2014 Normal	2014 Late
DTF	mean	58.4	50.3	52.5	55.6	47.3
	CV(%)	1.9	4.0	4.0	3.0	3.5
DTFT	mean	79.9	67.4	73.5	74.0	62.5
	CV(%)	4.7	5.9	8.1	3.9	2.5
DOF	mean	21.5	17.1	21.0	18.6	15.3
	CV(%)	17.2	21.0	26.2	15.1	12.5
Node	mean	18.8	20.4	20.9	20.9	20.1
	CV(%)	14.6	10.1	9.5	9.2	10.6
Rnode	mean	7.6	5.4	4.5	6.2	6.4
	CV(%)	20.6	26.3	25.7	15.6	15.5
Pod	mean	5.9	7.0	6.7	7.4	7.2
	CV(%)	28.0	24.7	23.7	16.9	20.0
Seed	mean	*	4.7	4.9	4.8	5.1
	CV(%)	*	17.3	14.4	14.8	12.5
SSW	mean	*	0.271	0.277	0.238	0.223
(g seed^{-1})	CV(%)	*	10.8	10.3	9.4	14.6
Yield	mean	464.7	554.0	694.8	473.8	425.7
(g m^{-2})	CV(%)	17.6	22.0	26.4	19.2	20.0

Notes: DTF: days to flowering (from sowing); DTFT: days to flowering termination; DOF: duration of flowering; Node: total node number per plant; Rnode: reproductive node number on the main-stem; Pod: pod number on the main-stem; seed: seed number per pod; SSW: single seed weight (g seed^{-1}); Yield: grain yield (g m^{-2}), *: value not measured in that year.

The mean, standard deviation (SD), minimum and maximum of nine traits for the 107 RIL members of PR-11, and the means of the parental cultivars based on 2013/14 Normal dataset and 2014 Late seeding date dataset are shown in Table 4.5 and Table 4.6. In addition, the same information of the RILs population based on the 2012 Normal dataset is provided in Appendix B.

Under normal seeding environments, CDC Centennial appeared to flower later than CDC Sage with a longer duration of flowering. CDC Centennial had greater pod numbers on the main-stem, single seed weight and grain yield than CDC Sage, whereas CDC Centennial had a lower seed number per pod than CDC Sage. No differences in node number and reproductive node number per plant were observed in these two parental lines.

At late seeding, CDC Centennial did not demonstrate any superiority to CDC Sage in terms of the measured traits. Still, the late seeding experiment was only carried out at one location in one year, and experiments in more years and at more locations would be needed to verify the current results.

Table 4.5 Mean, standard deviation (SD), minimum and maximum of nine traits for 107 RILs of CDC Centennial X CDC Sage population and the means of the parental cultivars based on the 2013/14 Normal dataset.

CDC Centennial X CDC Sage population					Parental cultivars	
					Mean	
Variable	Mean	Std Dev	Minimum	Maximum	Centennial	Sage
DTF	52.8	1.54	49.6	57.0	54.1	52.6
DTFT	71.6	2.70	66.1	77.6	74.1	70.6
DOF	18.8	2.20	13.7	23.7	20.0	17.9
Node	18.8	2.02	14.5	23.8	21.0	19.5
Rnode	5.4	0.57	4.2	7.0	5.3	5.4
Pod	7.0	0.78	5.5	9.0	7.5	6.7
Seed	4.8	0.38	4.0	5.7	4.4	5.4
SSW	0.270	0.0221	0.230	0.324	0.303	0.232
Yield	574.2	80.50	376.3	740.7	606.2	513.3

Notes: For explanation of DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW and Yield, refer to Table 4.4. 2013/14 Normal dataset: data collected in 2013 and 2014 at normal seeding dates.

Table 4.6 Mean, standard deviation (SD), minimum and maximum of nine traits for 107 RILs of CDC Centennial X CDC Sage population and the means of the parental cultivars based on the 2014 Late dataset.

CDC Centennial X CDC Sage population					Parental cultivars	
					Mean	
Variable	Mean	Std Dev	Minimum	Maximum	Centennial	Sage
DTF	47.2	1.46	44.0	51.0	47.3	46.4
DTFT	62.4	1.18	60.0	66.3	62.5	62.0
DOF	15.2	1.55	11.5	19.0	15.0	16.0
Node	20.1	1.77	15.5	24.5	18.6	20.3
Rnode	6.4	0.75	4.6	9.0	5.9	6.6
Pod	7.2	1.13	4.9	10.5	6.9	6.9
Seed	5.1	0.51	3.6	6.0	4.6	5.7
SSW	0.224	0.0249	0.163	0.344	0.240	0.198
Yield	425.7	69.3	257.7	559.0	457.0	446.3

Notes: For explanation of DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW and Yield, refer to Table 4.4.

4.3 DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW and yield

4.3.1 DTF, DTFT, DOF, Node, Rnode, Pod and Yield in 2012 and 2013 at Rosthern

The analysis of variance of DTF, DTFT, DOF, Node, Rnode, Pod and Yield in the RILs population evaluated in 2012 and 2013 at Rosthern is provided in Table 4.7. Genotypes varied significantly in all seven traits. Significant differences in DTF, DTFT, Node, Rnode and grain yield were observed between 2012 and 2013 at Rosthern. The genotype-by-year interaction had significant effects in all traits except for Node and yield.

Table 4.7.1 Analysis of variance of DTF, DTFT, DOF, Node and Rnode in PR-11 from 2012 and 2013 data at Rosthern.

		F values				
Effect	numDF	DTF	DTFT	DOF	Node	Rnode
Year	1	385.71**	235.14***	1.84 NS	92.38*	666.3***
Genotype	106	2.27***	1.59**	1.42*	1.32*	1.42*
Genotype×Year	106	1.97***	1.66***	1.37*	1.12 NS	1.63**

Notes: For explanation of DTF, DTFT, DOF, Node and Rnode, refer to Table 4.4.
NS: not significant; *: significant at P≤ 0.05; **: significant at P≤ 0.01; ***: significant at P≤ 0.001.

Table 4.7.2 Analysis of variance of Pod and Yield in PR-11 from 2012 and 2013 data at Rosthern.

		F Values	
Effect	numDF	Pod	Yield
Year	1	10.89 NS	369.34***
Genotype	106	1.42*	2.04***
Genotype×Year	106	1.47**	1.20 NS

Notes: Pod: pod number on the main stem; Yield: grain yield; NS: not significant; *: significant at P≤ 0.05; **: significant at P≤ 0.01; ***: significant at P≤ 0.001.
Seed number per pod and single seed weight were not measured in 2012 at Rosthern**.**

4.3.2 DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW and yield in 2013 at Saskatoon and Rosthern

Table 4.8 provides analyses of variance for DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW and yield with mean square values and significance in the RIL population of CDC Centennial X CDC Sage evaluated at Saskatoon and Rosthern in 2013. Genotypes significantly differed in all traits except for Rnode. Locations had significant differences in DTF, FTFT, DOF, Seed, SSW and grain yield. No significant effect of the interaction between genotype and location was observed in any of the nine traits.

Table 4.8.1 Analysis of Variance of DTF, DTFT, DOF, Node and Rnode in PR-11 evaluated in 2013 at both Saskatoon and Rosthern.

		F values				
Effect	numDF	DTF	DTFT	DOF	Node	Rnode
Location	1	246.7***	210.23***	64.49*	9.24 NS	16.08 NS
Genotype	106	5.00***	2.38***	1.72***	1.86***	1.26 NS
Genotype ×Location	106	1.00 NS	0.92 NS	0.93 NS	1.06 NS	1.16 NS

Notes: For explanation of DTF, DTFT, DOF, Node and Rnode, refer to Table 4.4.
NS: not significant; *: significant at l P≤ 0.05; **: significant at P≤ 0.01; ***: significant at P≤ 0.001.

Table 4.8.2 Analysis of Variance of Pod, Seed, SSW and Yield in PR-11 evaluated in 2013 at both Saskatoon and Rosthern.

		F Values			
Effect	numDF	Pod	Seed	SSW	Yield
Location	1	0.79 NS	6.02*	11.23**	117.2***
Genotype	106	1.33*	1.33*	4.33***	2.23***
Genotype ×Location	106	1.01 NS	1.13 NS	1.19 NS	1.12 NS

Notes: for explanation of Pod, Seed, SSW and grain yield, refer to Table 4.4.

4.3.3 DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW and yield in 2013 and 2014 at Saskatoon

Table 4.9 provides analyses of variances for DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW and yield with mean square values and significance level in the RIL population evaluated in 2013 and 2014 at Saskatoon. Genotypes differed significantly in all nine traits. DTF, DTFT, Node, Rnode, SSW and the grain yield showed significantly different between 2013 and 2014. The significant effect of genotype-by-year interaction was only observed for Rnode.

Table 4.9.1 Analysis of variance of DTF, DTFT, DOF, Node and Rnode in PR-11 from 2013 and 2014 data at Saskatoon.

		F values				
Effect	numDF	DTF	DTFT	DOF	Node	Rnode
Year	1	982.11**	116.56**	4.64 NS	9.77**	21.5*
Genotype	106	10.03***	3.29***	2.42***	2.22***	1.70***
Genotype ×Year	106	1.26 NS	1.33 NS	1.26 NS	1.01 NS	1.42*

Notes: For explanation of DTF, DTFT, DOF, Node and Rnode, refer to Table 4.4.
NS: not significant; *: significant at P≤ 0.05; **: significant at P≤ 0.01; ***: significant at P≤ 0.001.

Table 4.9.2 Analysis of variance of Pod, Seed, SSW and Yield in PR-11 from 2013 and 2014 data at Saskatoon.

		F Values			
Effect	numDF	Pod	Seed	SSW	Yield
Year	1	0.95 NS	0.71 NS	128.5**	51.35*
Genotype	106	1.45*	1.77***	4.57***	2.80***
Genotype ×Year	106	0.97 NS	1.04 NS	0.95 NS	1.20 NS

Notes: For explanation of Pod, Seed, SSW and yield, refer to Table 4.4.

4.3.4 DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW and Yield between two seeding dates in 2014

Analysis of variance for DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW and yield in RILs between two seeding dates in 2014 are shown in Table 4.10. Genotypes differed significantly for all traits. In addition, DTF, DTFT, DOF and Seed were significantly different between two seeding dates. The interaction between genotype and seeding date was significant for DTF and DOF.

Table 4.10.1 Analysis of variance of DTF, DTFT, DOF, Node and Rnode in PR-11 between normal and late seeding dates (Trt) in 2014.

		F values				
Effect	numDF	DTF	DTFT	DOF	Node	Rnode
Trt	1	956.39**	528.7**	37.99**	9.44 NS	1.57 NS
Genotype	106	9.32***	2.86***	2.60***	2.88***	1.94***
Genotype×Trt	106	1.82***	1.24 NS	1.39*	1.10 NS	0.96 NS

Notes: For explanation of DTF, DTFT, DOF, Node and Rnode, refer to Table 4.4.
NS: not significant; *: significant at l P≤ 0.05; **: significant at P≤ 0.01; ***: significant at P≤ 0.001.

Table 4.10.2 Analysis of variance of Pod, Seed, SSW and Yield in PR-11 between normal and late seeding dates in 2014.

		F values			
Effect	numDF	Pod	Seed	SSW	Yield
Trt	1	3.28 NS	45.61*	5.37 NS	13.15 NS
Genotype	106	2.17***	1.98***	2.86***	2.49***
Genotype×Trt	106	0.92 NS	1.06 NS	1.09 NS	0.97 NS

Notes: for explanation of Pod, Seed, SSW and grain yield, refer to Table 4.4.
NS: not significant; *: significant at P≤ 0.05; **: significant at P≤ 0.01; ***: significant at P≤ 0.001.

4.4 Selection

Based on yield performance, the top eight and bottom eight yielding lines were selected at the 2013/14 normal seeding plots (as I stated at the statistical analysis section 3.5.1 in chapter 3 “materials and method”, only RILs evaluated from the 2013/14 Normal dataset were regarded as the best representative RILs at normal seeding) and 2014 late seeding plots, respectively. Three common top yield lines (PR-11-2, PR-11-88 and PR-11-91) and five common bottom yield lines (PR-11-20, PR-11-38, PR-11-64, PR-11-73 and PR-11-90) were found under both normal and late seeding environments. The differences between the top and bottom yield groups at normal seeding date were mainly due to the differences in days to flowering (DTF), days to flowering termination (DTFT) and the duration of flowering (DOF; Table 4.11); that is, top yielding lines tended to have a relatively late start of flowering and a long duration of flowering compared to the bottom yielding lines. No significant differences were observed in pod number (Pod), seed number per pod (Seed) and single seed weight (SSW; Appendix N). Likewise, in the late seeding experiment, the highest yielding group tended to have the characteristics of a later onset of flowering and a longer flowering duration than the lowest yielding group. But no difference in the mean between top and bottom yield group for each trait was bigger than the least significant difference value (LSD) of the RIL population (Table 4.11, Appendix N). Still, more years’ data are needed to confirm the validity of this finding.

Table 4.11 The greatest (top) and smallest (bottom) yielding genotypes in the RIL population under the 2013 and 2014 normal seeding environments and the 2014 late seeding environment with DTF, DTFT and DOF of each line.

2013 & 2014 Normal				2014 Late			
Parental lines	DTF	DTFT	DOF	Parental lines	DTF	DTFT	DOF
CDC Centennial	54.1	74.1	20.0	CDC Centennial	47.3	62.5	15.0
CDC Sage	52.6	70.6	17.9	CDC Sage	46.4	62.0	16.0
Mean of the RIL population	52.8	71.6	18.8	Mean of the RIL population	47.2	62.4	15.2
High yield lines	DTF	DTFT	DOF	High yield lines	DTF	DTFT	DOF
PR-11-2	53.8	74.3	20.6	PR-11-2	48.2	66.3	17.8
PR-11-88	54.1	70.3	16.3	PR-11-88	49.0	64.0	15.5
PR-11-91	54.2	75.2	21.0	PR-11-91	49.0	65.0	16.0
PR-11-54	52.0	74.8	22.8	PR-11-7	51.0	64.3	13.3
PR-11-67	50.6	73.2	22.6	PR-11-15	48.3	63.5	15.3
PR-11-70	57.0	75.8	18.8	PR-11-18	45.8	63.0	17.3
PR-11-83	52.5	71.9	19.4	PR-11-29	48.0	62.0	14.0
PR-11-98	55.1	77.6	22.5	PR-11-44	46.3	63.5	17.3
Mean	53.6	74.1	20.5	Mean	48.2	63.9	15.8
Low yield lines	DTF	DTFT	DOF	Low yield lines	DTF	DTFT	DOF
PR-11-20	51.1	69.6	18.5	PR-11-20	45.8	61.3	15.5
PR-11-38	49.7	66.7	17.0	PR-11-38	46.5	60.8	14.3
PR-11-64	51.8	67.0	15.2	PR-11-64	47.3	63.3	16.0
PR-11-73	50.5	66.2	15.7	PR-11-73	45.3	60.5	15.3
PR-11-90	53.0	70.9	17.9	PR-11-90	47.3	62.8	15.5
PR-11-23	50.3	67.5	17.2	PR-11-27	46.8	60.8	14.0
PR-11-31	50.0	67.1	17.1	PR-11-58	48.3	61.5	13.3
PR-11-106	52.1	67.9	15.8	PR-11-80	47.0	62.5	15.5
Mean	51.1	67.9	16.8	Mean	46.8	61.7	14.9
LSD	3.1	5.7	4.7		2.1	2.7	3.1

4.5 Correlations

4.5.1 Correlations based on 2013/14 Normal seeding dataset

Table 4.12 shows the correlation coefficients among nine traits based on the averaged data of 2013 and 2014 at normal seeding dates. DTF was positively correlated with DTFT ($r=0.58$, $P<0.001$), Seed ($r=0.32$, $P<0.001$) and grain yield ($r=0.36$, $P<0.001$), and negatively correlated with Pod ($r=-0.27$, $P<0.01$). DTFT was positively correlated with DOF ($r=0.82$, $P<0.001$), Node ($r=0.23$, $P<0.05$), Rnode ($r=0.24$, $P<0.05$), and yield ($r=0.47$, $P<0.001$). DOF was positively correlated with Node ($r=0.19$, $P<0.05$), Rnode ($r=0.31$, $P<0.01$), Pod ($r=0.18$, $P<0.05$) and yield ($r=0.33$, $P<0.001$), but negatively correlated with Seed ($r=-0.27$, $P<0.01$). Node was not correlated with any of other eight traits. Rnode was positively correlated with Pod ($r=0.58$, $P<0.001$) and negatively correlated with SSW ($r=-0.29$, $P<0.01$). Pod was negatively correlated with both Seed ($r=-0.35$, $P<0.001$) and SSW ($r=-0.21$, $P<0.05$). In addition, the correlation coefficients among the measured traits based on the 2012 Normal dataset is shown in Appendix L.

Table 4.12 Correlations between DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW and plot yield in PR-11 based on 2013/14 Normal seeding dataset.

	DTF	DTFT	DOF	Node	Rnode	Pod	Seed	SSW	Yield
DTF	-	0.58***	0.01	0.12	-0.03	-0.27**	0.32**	0.12	0.36***
DTFT		-	0.82**	0.23*	0.24*	-0.01	-0.04	0.13	0.47***
DOF			-	0.19*	0.31**	0.18*	-0.27**	0.08	0.33*
Node				-	0.09	0.10	0.04	-0.02	-0.01
Rnode					-	0.58***	-0.29**	0.02	0.13
Pod						-	-0.35***	-0.21*	0.01
Seed							-	-0.15	0.04
SSW								-	-0.01
Yield									-

Notes: For explanation of DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW and yield, refer to Table 4.4.
*: significant at P≤ 0.05; **: significant at P≤ 0.01; ***: significant at P≤ 0.001.

4.5.2 Correlations at late seeding date in 2014

Under late seeding in 2014, DTF was positively related with DTFT (r=0.29, P<0.01), negatively related with DOF (r=-0.7, P<0.001) (Table 4.13). DTFT was positively correlated with DOF (r=0.42, P<0.001), Rnode (r=0.2, P<0.05) and Yield (r=0.29, P<0.01). DOF was positively correlated with Rnode (r=0.23, P<0.05), Pod (r=0.20, P<0.05), negatively correlated with Seed (r=-0.21, P<0.05). Node was positively correlated with Rnode (r=0.54, P<0.001), Pod (r=0.63, P<0.001), yield (r=0.25, P<0.01), but negatively correlated with Seed (r=-0.24, P<0.05). Rnode was positively correlated with pod (r=0.74, P<0.001). Pod was positively correlated with yield (r=0.21, P<0.05), and negatively correlated with Seed (r=-0.28, P<0.05).

Table 4.13 Correlations between DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW and plot yield in PR-11 based on 2014 Late dataset.

	DTF	DTFT	DOF	Node	Rnode	Pod	Seed	SSW	Yield
DTF	-	0.29***	0.70***	0.08	-0.01	0.05**	0.11	0.04	0.14
DTFT		-	0.42**	0.11	0.20*	0.10	0.13*	0.01	0.29***
DOF			-	0.06	0.23*	0.20*	-0.21*	-0.07	0.08
Node				-	0.54***	0.63***	-0.24*	-0.10	0.25**
Rnode					-	0.74***	0.01	0.02	0.08
Pod						-	-0.28*	-0.14*	0.21*
Seed							-	0.23	0.19
SSW								-	-0.05
Yield									-

Notes For explanation of DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW and yield, refers to Table 4.4.
*: significant at P≤ 0.05; **: significant at P≤ 0.01; ***: significant at P≤ 0.001.

4.5.3 Correlations among temperature, flowering and yield

The only significant correlation was observed between yield and DOF (r=0.97, P<0.01; Table 4.14). Although no significant correlations were shown between daily maximum temperature and DOF, daily maximum temperature and yield, there was a clear trend that daily maximum temperature was negatively correlated with DOF (r=-0.69, P=0.10) and yield, especially yield (r=-0.81, P=0.06).

Table 4.14 Correlations between the mean of daily maximum temperature, the mean of daily temperature, frequency of high temperature, duration of flowering (DOF) and plot yield among location-years.

	Mean of max	Daily average	Frequency	DOF	Yield
Mean of max	-	-	-	-0.69	-0.81
Daily average		-	-	-0.61	-0.75
Frequency			-	-0.39	-0.56
DOF				-	0.97**
Yield					-

Notes: Mean of max: the mean of daily maximum temperature; Daily average: average daily temperature; Frequency: frequency of daily maximum temperature over 27 ℃; DOF: duration of flowering; **: significant at P≤ 0.01.
Temperature information was based on the average DOF of RILs at each location-year.

4.5.4 Path coefficient analysis of four yield component traits on main-stem yield under normal and late seeding environments

Path analysis (also known as structural equation modelling) was conducted in order to discover the cause-and-effect relationship between yield components and seed yield. Results revealed pod numbers on the main-stem (Pod), seed numbers per pod (Seed) and single seed weight (SSW) had positive effects on seed yield on the main-stem, Rnode had a negative effect on the main-stem seed yield, regardless of sowing dates (Fig 4.1, 4.2). The results demonstrated that the variation of seed yield on the main-stem was principally derived from Pod, followed by SSW and Seed, though the exact proportion of yield variation explained by each component was not exactly the same between the two seeding dates. However none of the main-stem yield under any of the two environments was significantly associated with its final plot yield, meaning that side-branches had a significant influence given that all the RILs had the same germination ability, and likely a similar stand establishment. Additionally, indirect effects were not accounted

for in the model, so the residual (unaccounted for) error would be inflated, which would diminish the relative effect of un-accounted for branches on yield.

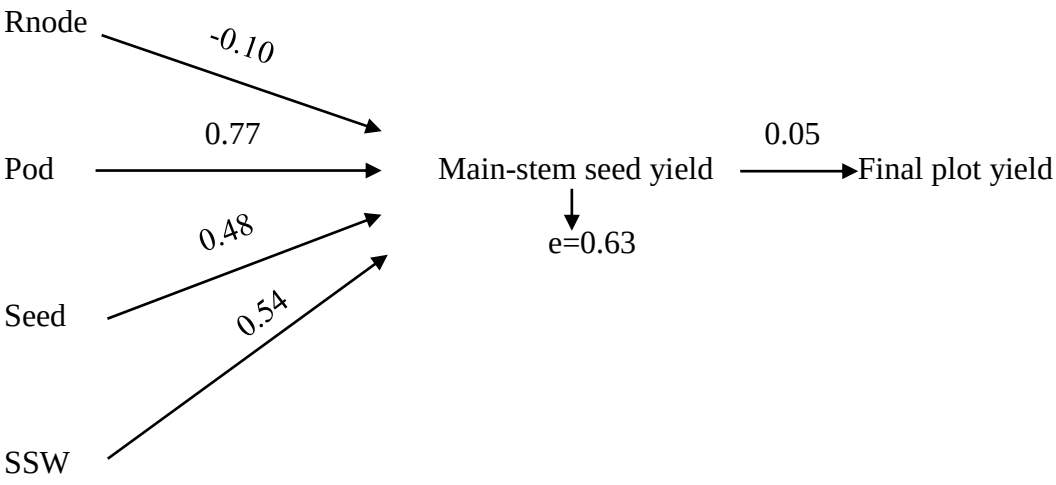


Figure 4.1 Path coefficient analysis of four yield component traits (Rnode, Pod, Seed and SSW) on main-stem seed yield under normal seeding date environment. The partial coefficients, r, are the numbers above the arrows, and the residual error unaccounted for error is e.
For the explanation of Rnode, Pod, Seed and SSW, refer to Table 4.4.

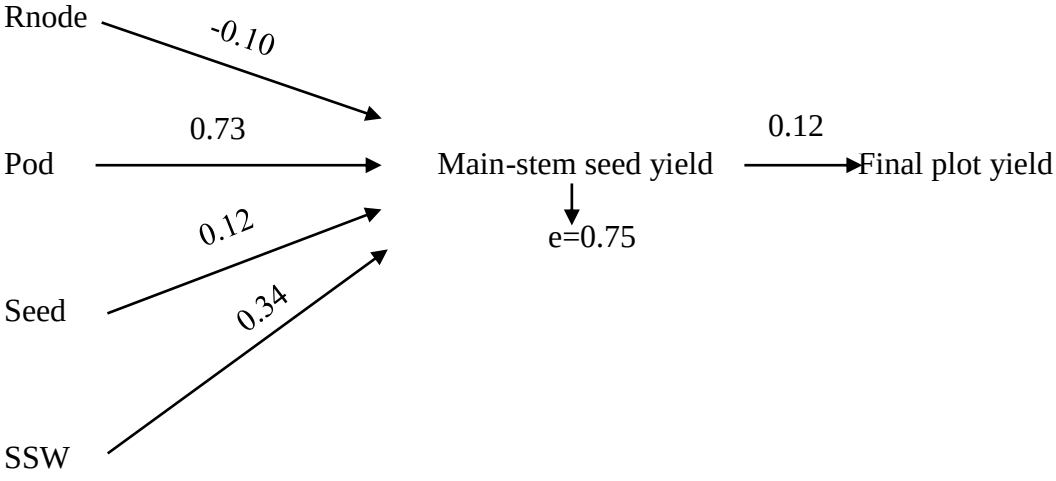


Figure 4.2 Path coefficient analysis of four yield component traits (Rnode, Pod, Seed and SSW) on main-stem seed yield under late seeding date environment. The partial coefficients, r, are the numbers above the arrows, and the residual error unaccounted for error is e.
For the explanation of Rnode, Pod, Seed and SSW, refer to Table 4.4.

4.6 Estimates of variance components and heritability

The heritability of DTF, DTFT, DOF, Pod, SSW and Yield for 107 RILs of CDC Centennial/ CDC Sage was stable between the combined data for 2013/14 at Saskatoon (Table 4.15) and combined data for Saskatoon/ Rosthern in 2013 (Table 4.16). Among these six traits, all traits except Pod showed high heritability, the highest was in DTF (h^2=0.84), followed by SSW (h^2=0.76), DTFT (h^2=0.60), Yield (h^2=0.54) and DOF (h^2=0.45). Pod showed only moderate heritability (h^2=0.29).

The heritability of DTF, DTFT, DOF, Node, Rnode, Pod and yield for 107 RILs of PR-11 grown at Rosthern across 2012/13 were low (Appendix M), due to the fact that the heterogeneity between 2012 and 2013 increased the variance of error dramatically and the significant interaction between genotype-by-year reduced the genotypic variance to a large extent.

Table 4.15.1 Estimates of the partial variance components and heritability calculation of DTF, DTFT, DOF, Node and Rnode for 107 RILs of CDC Centennial X CDC Sage grown at Saskatoon in 2013 and 2014.

Variance components	DTF	DTFT	DOF	Node	Rnode
σ^2_g	2.23±0.35	3.23±0.80	2.08±0.67	0.93±0.24	0.08±0.08
σ^2_{gy}	0.13±0.10	1.09±0.68	0.92±0.71	0	0.25±0.13
σ^2_e	1.02±0.10	6.61±0.64	7.17±0.70	3.05±0.24	1.12±0.11
σ^2_p	2.55	5.43	4.33	1.69	0.49
h^2	0.87	0.60	0.48	0.16	0.33

Notes: For explanation of DTF, DTFT, DOF, Node and Rnode, refer to Table 4.4.
σ^2_g: genotype variance; σ^2_{gy}: genotype by year interaction variance; σ^2_e: error variance; σ^2_p: phenotypic variance; h^2: broad-sense heritability. Up to 10%= low heritability; 20-30%= medium heritability; >30%= high heritability.

Table 4.15.2 Estimates of the partial variance components and heritability calculation of Pod, Seed, SSW and grain yield for 107 RILs of CDC Centennial X CDC Sage grown at Saskatoon in 2013 and 2014.

Variance components	Pod	Seed	SSW x10^3	Yield
σ^2_g	0.24±0.11	0.09±0.03	0.97±0.17	3070±804.8
σ^2_{gy}	0	0.01±0.04	0.01±0.02	775.8±737.2
σ^2_e	1.98±0.16	0.48±0.05	1.04±0.08	7699±747.8
σ^2_p	0.74	0.22	1.235	5383
h^2	0.33	0.42	0.79	0.57

Notes: For explanation of Pod, Seed, SSW and yield, refer to Table 4.4.
σ^2_g: genotype variance; σ^2_{gy}: genotype by year interaction variance; σ^2_e: error variance; σ^2_p: phenotypic variance; h^2: broad-sense heritability.

Table 4.16.1 Estimates of partial variance components and heritability calculation DTF, DTFT, DOF, Node and Rnode for 107 RILs of CDC Centennial X CDC Sage grown at Saskatoon and Rosthern in 2013.

Variance components	DTF	DTFT	DOF	Node	Rnode
σ^2_g	2.07±0.36	6.57±1.57	3.34±1.15	0.70±0.25	0.04±0.09
σ^2_{gy}	0.00±0.17	0.00	0.00	0.09±0.30	0.13±0.14
σ^2_e	2.07±0.20	18.211±1.44	18.05±1.43	3.42±0.33	1.49±0.15
σ^2_p	2.59	11.12	7.94	1.60	0.48
h^2	0.80	0.59	0.43	0.44	0.08

Notes: For explanation of DTF, DTFT, DOF, Node, Rnode, refer to Table 4.4.

σ^2_g: genotype variance; σ^2_{gy}: genotype by location interaction variance; σ^2_e: error variance; σ^2_p: phenotypic variance; h^2: broad-sense heritability.

Table 4.16.2 Estimates of partial variance components and heritability calculation of Pod, Seed, SSW and grain yield for 107 RILs of CDC Centennial X CDC Sage grown at Saskatoon and Rosthern in 2013.

Variance components	Pod	Seed	SSW x10^3	Yield
σ^2_g	0.20 ±0.15	0.02 ±0.03	0.93 ±0.18	5008.64 ±1552.00
σ^2_{gl}	0.04 ±0.22	0.04 ±0.05	0.10 ±0.11	1109.62 ±1647.92
σ^2_e	2.44 ±0.24	0.51 ±0.05	1.16 ±0.12	18110 ±1750.76
σ^2_p	0.83	0.17	1.27	10090.95
h^2	0.24	0.12	0.73	0.50

Notes: For explanation of Pod, Seed, SSW and yield, refer to Table 4.4.
σ^2_g: genotype variance; σ^2_{gl}: genotype by location interaction variance; σ^2_e: error variance; σ^2_p: phenotypic variance; h^2: broad-sense heritability.

4.7 Genotyping results

4.7.1 General features of the map

One hundred six RILs of PR-11 (only 106 lines were genotyped, line 83 was absent) and two replications of the parents CDC Centennial and CDC Sage were screened against a panel of 1536 GoldenGate markers developed by Sindhu et al (2014). Of the 1536 SNP markers, 377 markers showed polymorphism between CDC Centennial and CDC Sage. Among these markers, 369 markers generated clear segregating bands among the RILs.

A genetic linkage map with 8 linkage groups (LGs) was developed using these 369 markers. These 8 LGs were aligned with the 7 LGs previously published by Leonforte et al (2013) using 408 gene-based SNPs. LGV was divided into two independent segments identified as LGV-a and LGV-b respectively due to the lack of marker coverage. The graphical map is provided in Figure 4.3.

The total size of the map was 746.2 cM and the average distance between markers was 2.02 cM. The size of individual LG ranged from 1 cM (LGV-a) to 148 cM (LGIII) and the average density of markers varied from 0.33 marker per cM (LGI) to 6 markers per cM (LGV-a). The general features of each LG are summarized in Table 4.17.

Table 4.17 General features of genetic map using SNP markers based on 106 RILs of the CDC Centennial/CDC Sage population.

Linkage Groups	Size (cM)	Number of markers	Average marker distance (cM)
I	114.8	38	3.0
II	113.2	38	2.97
III	148	72	2.05
IV	115	65	1.77
V-a	1	6	0.17
V-b	34	17	2
VI	121.1	59	2.05
VII	99.9	74	1.35
Total	746.2	369	2.02

Note: cM=centiMorgan.

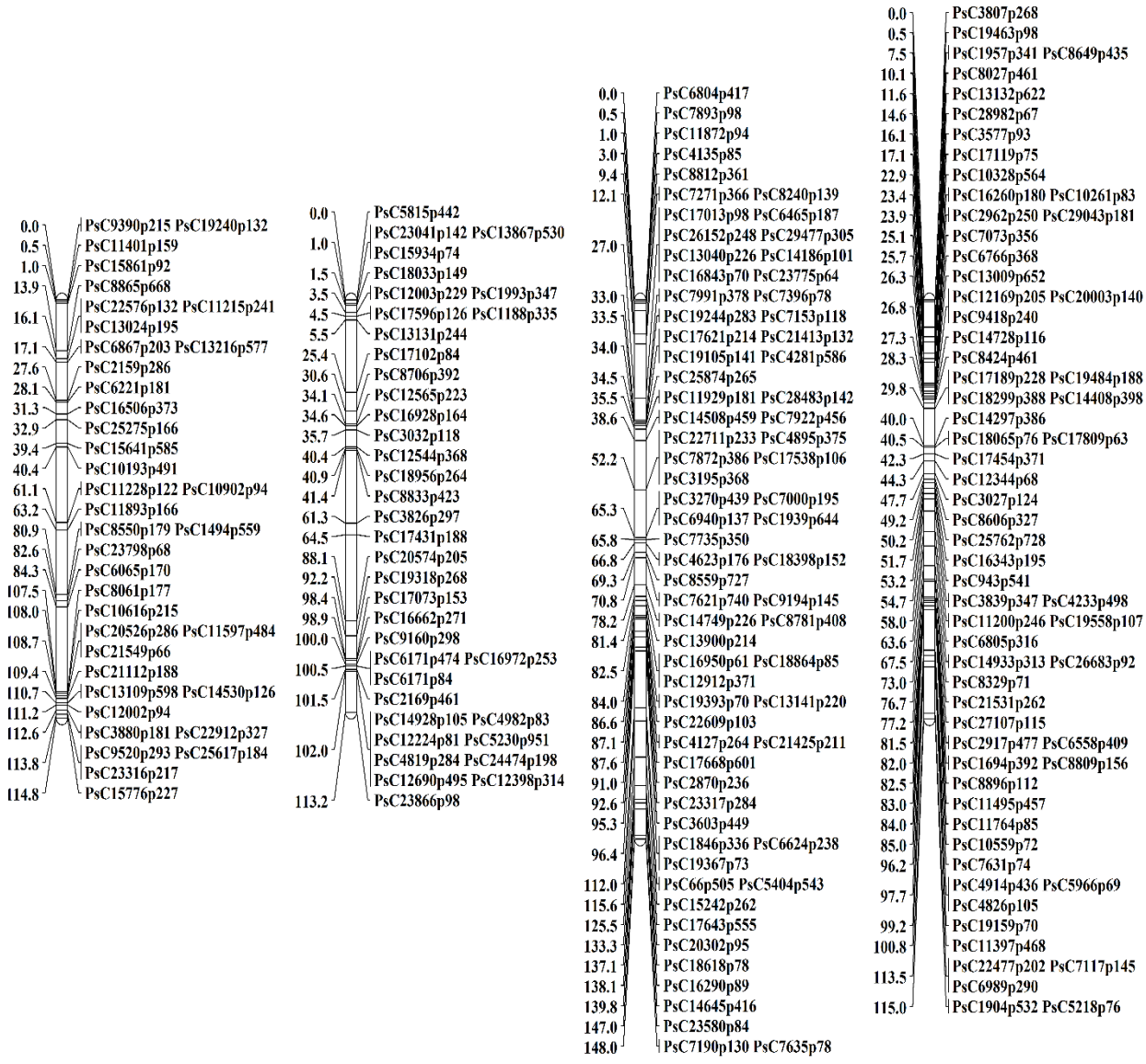
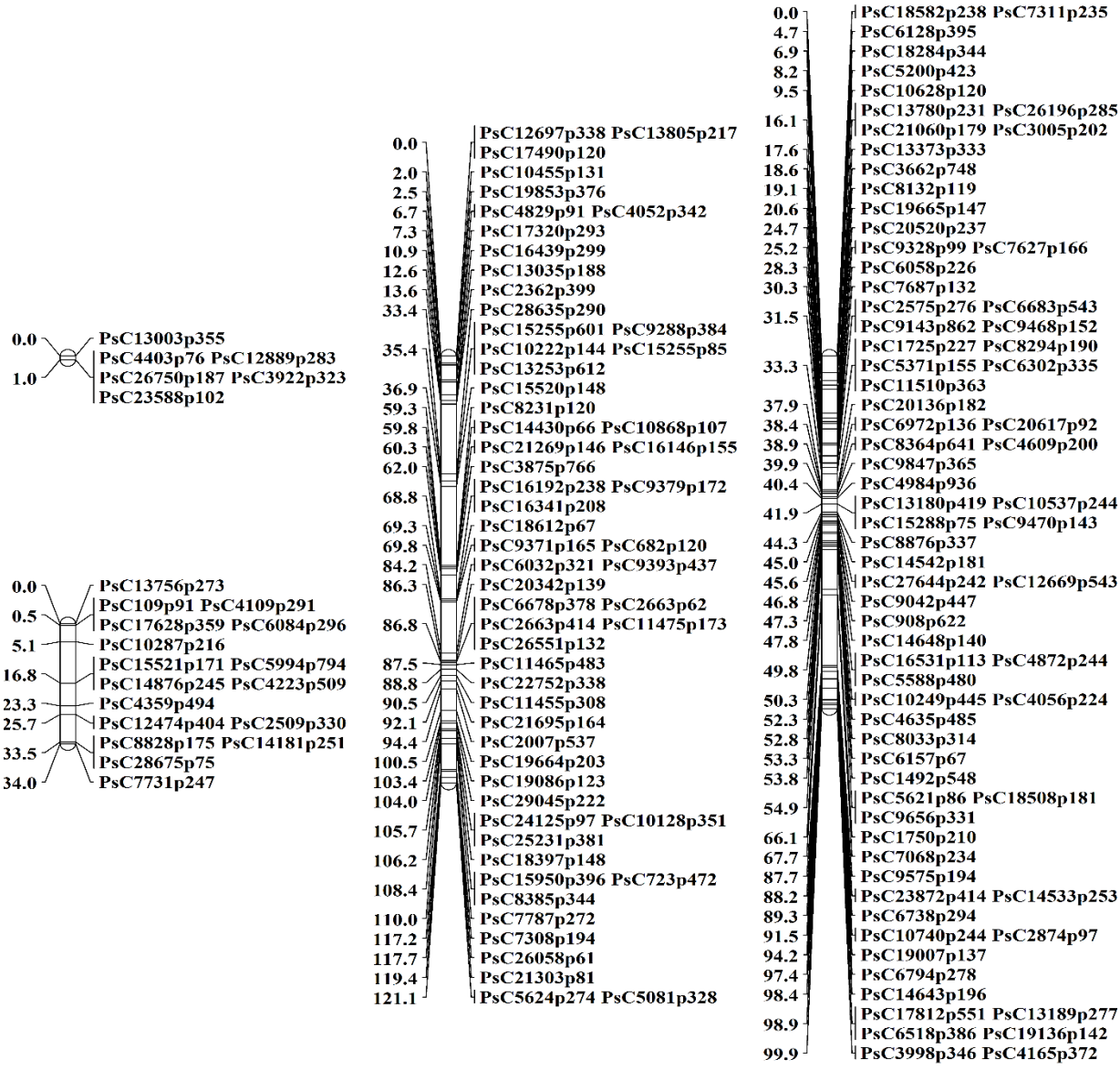


Figure 4.3 Genetic linkage map based on 369 SNP markers segregation on 106 RILs derived from PR-11 (CDC Centennial X CDC Sage).
LGI, LGII, LGIII, LGIV, LGV, LGVI and LGVII represent the linkage groups assigned to pea's seven chromosomes (given in parenthesis). The figures to the left of each linkage group bar represent the genetic distance (cM). Markers assigned to each linkage group are given to the right side of each linkage bar. LGV are divided into two segments (LGV-a, LGV-b) due to lack of anchored markers.

Continued Page 49



Continued Figure 4.3 Genetic linkage map based on 369 SNP markers segregation on 106 RILs derived from PR-11 (CDC Centennial X CDC Sage).

4.7.2 QTL analysis of phenotypic traits

4.7.2.1 QTL analysis based on 2013/14 Normal dataset

A total 14 QTLs linked with the traits were detected based on the combined data across two locations in 2013 and 2014 at normal seeding dates (Table 4.18 and Appendix O), six for flowering traits and eight for yield component traits. The QTL for DTF was on LGV with R^2=23.7. There were three QTLs associated with DTFT, one on LGIII (R^2=19), the second on LGIV (R^2=14), and the third on LGVI (R^2=11). Two QTLs were linked with DOF, one was on LGIII at the distance of 33cM with a LOD value of 7.43, the other was on LG IV at the distance of 82cM with a LOD value of 6.59. These two QTLs explained 42% of the phenotypic variation of DOF.

Among the eight QTLs associated with yield component traits, two were detected for each of Node, Seed and SSW, one for Pod, and one for Yield. No QTL was seen significantly correlated with Rnode. There were five QTLs on LGIII, each QTL for Node (R^2=24.7), Pod (R^2=14.7), Seed (R^2=18.3), SSW (R^2=6.4) and Yield (R^2=21.3), at the distance of 38.6, 50.6, 66.8, 70.8 and 33.5 respectively. Two QTLs (one for Seed with R^2=13.5, one for SSW with R^2=28.0) were located on LGIV. These two QTLs were at only 1cM distance between each other. On LGVII one QTL (91.5cM) showed significant association with Node, it had a LOD score of 3.6 and explained 14.7% of Node's phenotypic variation.

Table 4.18 Fourteen QTLs identified for phenotypic traits based on the 2013/14 Normal dataset for 106 RILs from the CDC Centennial X CDC Sage population.

Phenotype[a]	LG	Location (cM)	Maximum LOD Value	Closest Marker[b]	LOD[c]	$R^{2,d}$	Add[e]
DTF	VI	69.8	6.23	PsC9371p165	2.8	23.7	-0.76
DTFT	III	33	7.68	PsC7991p378	3.0	19.0	1.21
DTFT	IV	82	5.9	PsC1694p391	3.0	14.0	-0.94
DTFT	VI	69.8	4.78	PsC9737p165	3.0	11.0	-1.03
DOF	III	33	7.43	PsC7991p378	2.9	22.8	1.06
DOF	IV	82	6.59	PsC1694p392	2.9	19.8	-0.99
Node	III	38.6	7.74	PsC14508p459	2.9	24.7	0.95
Node	VII	91.5	3.65	PsC22711p233	2.8	14.7	0.26
Pod	III	50.6	3.65	PsC4895p375	2.9	14.7	0.32
Seed	III	66.8	5.38	PsC4895p375	3.0	18.3	-0.16
Seed	IV	82.5	4.08	PsC8896p112	3.0	13.5	0.14
SSW	III	70.8	3.34	PsC7621p740	3.0	6.4	-0.001
SSW	IV	81.5	12.01	PsC2917p477	3.0	28.0	-0.01
Yield	III	33.5	5.52	PsC19244p283	3.0	21.3	37.04

[a]. For explanation of DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW, Yield, refer to Table 4.4.
[b]: Closest marker to the identified QTL with the maximum LOD score.
[c]: Threshold level to declare a QTL significant was determined by performing the 1000 permutation test. [d]: Percentage of total variability explained by the QTL detected for the trait. [e]: additive effect of detected QTL for phenotypic traits (a positive value means CDC Centennial increased the value of the trait, a negative value means CDC Centennial decreased the value of the trait).

4.7.2.2 QTL analysis based on 2014 Late dataset

Eight QTLs were found among 106 RILs grown at late seeding date in 2014 (Table 4.19 and Appendix P), three for DTF, one for DOF, Node, Pod, SSW and Yield, respectively. Among the three QTLs with DTF, one was on LGII at the distance of 40.9 cM which explained 12.4% phenotypic variation. Another was on LGIII at the distance of 65.3 explaining 14.6% phenotypic variation, and the third one was on LGVI at the distance of 69.8cM (R^2=15.6). The QTLs of Pod and Yield were located on LGIII, at the distance of 59.2 and 52.2cM, respectively. The QTL for

Pod explained 16.9% of its phenotypic variation and the QTL for Yield explained 12.3% of its phenotypic variation. The QTL for SSW was on LGIV (80.2cM) and was responsible for 11.9% of its phenotypic variation. On LG VII, two QTLs were detected, one was for DOF at 46.8cM (R^2=13.6), the other was for Node at 98.4cM (R^2=10.3).

Table 4.19 Eight QTLs identified for phenotypic traits based on the 2014 Late dataset for 106 RILs from CDC Centennial X CDC Sage population.

Phenotype[a]	LG	Location (cM)	Maximum LOD Value	Closest Marker[b]	LOD[c]	$R^{2,d}$	Add[e]
DTF	II	40.9	4.89	PsC18956p264	2.8	12.4	0.53
DTF	III	65.3	5.62	PsC3270p439	2.8	14.6	-0.61
DTF	VI	69.8	5.97	PsC9371p165	2.8	15.6	-0.60
DOF	VII	46.8	3.37	PsC9042p447	2.8	13.6	-0.58
Node	VII	98.4	3.0	P Sc14643p196	2.9	10.3	-0.59
Pod	III	59.2	5.45	PsC3270p439	3.0	16.9	0.47
SSW	IV	80.2	2.98	PsC2917p477	2.9	11.9	-0.00
Yield	III	52.2	3.02	PsC22711p233	2.9	12.3	24.70

a. For explanation of DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW and Yield, refer to Table 4.4.
b: Closest marker to the identified QTL with the maximum LOD score.
c: Threshold level to declare a QTL significant was determined by performing the 1000 permutation test. d: Percentage of total variability explained by the QTL detected for the trait. e: additive effect of the detected QTL for phenotypic traits. (a positive value means CDC Centennial increased the value of the trait, a negative value means CDC Centennial decreased the value of the trait).

5.0 DISCUSSION

5.1 Climatic effects on this experiment

The experiment was conducted at two locations (Saskatoon and Rosthern) over three years (2012-2014). Both the average temperature of the growing season and the vegetative stage were similar over three years across these two sites (Table 4.1, 4.3), they did not exceed the maximum mean seasonal temperature threshold (17.5 ℃) for pea yield reduction (Bueckert et al, 2015), and they were in the optimal temperature range for pea growth (Mahoney, 1991). However, the daily mean temperature and daily maximum temperature at anthesis varied among station-years (Table 4.2). The average daily temperature ranged from 16.8 ℃ (2013 Saskatoon) to 20.4 ℃ (2012 Rosthern), the average daily maximum temperature varied from 22.8 ℃ (2013 Rothern) to 26 ℃ (2012 Rosthern). Even at the most heat sensitive stage (Zinn and Tunc-Ozdemir, 2010), the mean daily temperature still sat within the ideal range, whereas the mean daily maximum temperature at some location-years (2012 Rosthern, 2014 Saskatoon late seeding plot) surpassed the broadly accepted upper optimal temperature threshold 25 ℃ (Pumphrey and Raming, 1990), and days with the maximum temperature over 27 ℃ occurred at all locations over three years. These two facts verified the existence of heat stress. Further, when studying the relationships between the daily maximum temperature and the final grain yield and the flowering duration of the RILs population (Table 4.14), it showed that yield and flowering duration were negatively correlated with the daily maximum temperature (r=-0.81, r=-0.69), in agreement with Bueckert et al (2015), so when the mean maximum temperature was over 25 ℃, the shorter the pea's reproductive stage became in dryland conditions.

It is difficult to simulate heat stress on a field basis in glasshouses or growth chambers. One well-accepted and economical method that was used in this study is to plant crops at different

dates in hope of receiving heat stress at different stages in the plant's cycle, and this method has has been used in various crop breeding programs including pea (French, 1990; Munier-Jolain and Carrou é e, 2010), chickpea (Krishnamurthy et al, 2011), and summer *Brassica* (Morrison and Stewart, 2002).

In 2014, the RIL population (PR-11) was seeded at two different dates (normal and late), and late seeding induced more heat stress. During flowering both the daily maximum temperature and frequency of days with maximum temperature ≥27 ℃ were higher in late seeded plots than those in normal seeded plots (Table 4.2), which proved the effectiveness of this method. Nevertheless, a late seeding date in the study still did not bring sufficient stress to cause detrimental effects on yield and yield-related traits, because RILs at different seeding dates failed to demonstrate significant differences in yield components and final grain yield (Table 4.10). Maybe more seeding dates, or more years of studying are needed to study field-based heat stress on a large scale.

5.2 Phenotypic trait assessments

Grain yield per plant can be expressed as the function of the number of reproductive nodes per plant (Rnode), pod number per reproductive node (Pod), seed number per pod (Seed) and single seed weight (SSW) in a multiplicative way. The analysis of variances of these traits in this study revealed the existence of genotypic variation among the yield component traits (Table 4.7-4.10). Corresponding evidence of the high degree of genetic variability in field pea related to yield components has already been reported by Sharma et al (2003), Ranjan et al (2006), Singh et al (2011) and Kumar et al (2013).

The analyses of associations among the four yield components (Rnode, Pod, Seed and SSW) turned out to imply that they were only weakly associated with one another. Only Pod

showed a significant positive association with Rnode, and negative correlations with Seed and SSW under both normal and late seeding environments (Table 4.12, 4.13). Earlier reports in this respect were contradictory, one study was in agreement with our results (Krajewski et al, 2012), but another research pointed out more significant correlations existed among yield components (Fikreselassie, 2012). Also when comparing the results in this research with those by other authors, one thing needs to be noted is that unlike previous researches, this study only recorded the yield components on the main stem instead of the whole plant. Likely the increase in pod number per plant (Pod) was offset by the decrease in seed number per pod (Seed), and the negative relationship between Pod and Seed was not only displayed in our study but also in other research (Moot and McNeil 1995; Krajewski et al, 2012). This reason might be attributed to competition for assimilates during reproductive growth, or in particular when environmental stresses occur in the reproductive stage.

Further, the path coefficient analysis (multiplying the ordinary regression coefficient by the standard deviation of the corresponding variable) was carried out to discover the relative contribution of yield components to the grain yield on the main stem under normal and late seeding environments. Both results revealed that the variance of the main-stem seed yield primarily derived from the variance of Pod number (Fig 4.1, 4.2). Consistent results have been reported by French (1990), Ayaz et al (2004) and Singh and Singh (2005), which stated that seed yield per plant was most positively correlated with the number of pods per plant. As well, Sarawat et al (1994) concluded that grain yield heterosis was mainly due to more pods per plant in hybrids. The variation in seed yield on the main stem appeared not to be the cause of the major variation in final grain yield on a plot scale (no significant correlation was observed between these two). Given that the emergence rate among RILs was similar, this finding indicated that the final grain yield was derived also from seed yield on the branches, or indirect

effects that were not measured in the model. The importance of the contribution of the basal branches on yield has already been emphasized by Munier-Jolain and Carrou é (2010) and Singh et al (2011). However, studies regarding the initiation of branches are less well documented than for the main stem, and previous authors concluded that the ability to produce basal branches mainly depended on pea genotype and plant density (Spies et al, 2010). Besides yield components, phenological traits like days to flowering (DTF) and duration of flowering (DOF) also affect grain yield in pea. DTF was positively associated with grain yield in this study, as well as in others (Timmerman-Vaughan et al, 2005; Singh et al, 2011; Bueckert and Clarke, 2013). The reason might be that delayed onset of flowering may allow for greater assimilate production through vegetative development which could be used for the reproductive development.

Among the flowering and yield-related traits in this study, the broad-sense heritability of days to flowering (DTF) and single seed weight (SSW) were both fairly high (h^2>0.7), days to flowering termination (DTFT), duration of flowering (DOF) and grain yield showed moderately high broad-sense heritability (h^2 >0.5), whereas moderate to low heritability (h^2 ranged from 0.1 to 0.3) was found in Rnode, Pod and Seed (Table 4.15, 4.16). This finding was in agreement with previous publications (Timmerman-Vaughan et al, 2005; Singh et al, 2011; Fikreselassie, 2012).

5.3 Linkage map quality

The total coverage of the PR-11 linkage map was 746.2 cM (Table 4.17), which is in the range of the previous published SNP-derived maps for pea, ranging from 358.02 cM to 1916 cM (Deulvot et al, 2010; Leonforte et al, 2013; Duarte et al, 2014; Sindhu et al, 2014). It was especially in agreement with the consensus map by Sindhu et al (2014) covering 771.6 cM for

seven linkage groups based on five populations, because the same SNP markers were in use. The average distance between two markers in the map was 2.02 cM with the largest interval less than 15 cM. The inter-locus interval in the map was less than most of the published genetic maps for pea through SNP markers (i.e. 8.2 cM in Deulvot et al, 2010; 4.2 cM in Duarte et al, 2014), indicating a good quality and density of this map.

5.4 QTLs for flowering-related traits

Six and four QTLs related to flowering traits (DTF, DTFT, DOF) were identified using RILs sown at normal and late seeding plots, respectively (Table 4.18, 4.19).

A QTL for days to flowering (DTF) located on LGVI at the distance of 69.8 cM was detected under both normal and late sowing environments which accounted for 23.7% of its phenotypic variation in the normal seeding date experiment, and 15.6% at the late seeding date. Prioul et al (2004) based an analysis on F_2 –derived recombinant inbred lines from the cross of JI296 (a white- flowered, early flowering cultivar) X DP (a purple-flowered, late flowering cultivar), found three QTLs responsible for days to flowering, and one of these was situated at a similar position on LGVI as reported here. Fondevilla et al (2008) identified a different QTL for DTF on LGVI, which was 20 cM away from the one detected here. They also found a QTL on LGIII with a major effect (R^2=0.54) on DTF in their RIL population. This QTL was also reported to relate to earliness in a previous paper (Timmerman-Vaughan et al, 2004).

Two QTLs (one on LGIII, one on LGIV) for DOF at the normal seeding date shared the same genomic regions with the QTLs for DTFT, which validated the reported high correlation result between these two traits. The QTL on LGIII was also reported by Prioul et al (2004), who stated this region displayed a significant correlation with DTF as well as partial resistance to *Mycosphaerella pinodes*.

The other QTL for DTFT coincided with the QTL discussed above for DTF. The coincidence of QTLs for flowering related traits with those published by previous authors supported the validity of the QTLs detected here. However, no QTL with a major effect was identified (highest R^2 is 0.24 for DTF), which indicated that the control of flowering time is multi-genic. QTLs controlling DOF across different seeding environments were different, possibly indicating that different genetic mechanisms are involved, but further verification of QTL stability across late-sowing environments would be needed because late seeding experiment was only conducted at one location in one year.

5.5 QTLs for yield and yield component traits

As many as six and four QTLs revealed significant correlation with yield and yield component traits (Rnode, Pod, Seed and SSW) based on normal and late seeded RILs, respectively. The number of QTLs found were low compared to other publications (Timmerman-Vaughan et al, 1996; Irzykowska and Wolko, 2004; Tar'an et al, 2004; Timmerman-Vaughan et al, 2005; Krajewski et al, 2012). Part of the reason might be attributed to a lack of variation between the two parents regarding these traits. The loci found by RILs from the normal seeding date were different to those detected by the same RILs with the late seeding date, except for the QTL on the LGIV for SSW. The difference can be explained by either a consequence of environmental factors fluctuating across plots (Paterson et al, 1991) or some genes related with heat resistance (as RILs at late seeding date plots were considered to be more heat stressed) having pleiotropic effects over the QTLs for yield components in the normal environment. To my knowledge, no paper has yet reported any QTL analysis for late seeding or heat stress environment in pea, so no comparable reference is available. Still, more years of

assessment of late seeded RILs are needed to validate the detection of QTLs based on RILs from the 2014 late seeded environment.

Two QTLs for seed number per pod (one on LGIII, one on LGIV) coincided with the loci for single seed weight. Timmerman-Vaughan et al (2005) explained the coincidence of QTL as either the existence of a causal relationship, or that single genes underlying the QTL have pleiotropic effects, or the genomic regions associated with these QTLs carry groups of linked genes that affect yield components. A deeper understanding of the mechanisms or dissection of relevant genes at these loci are needed.

5.6 Comparison of the top and bottom yield groups in the RILs population

The top eight yielding lines were selected from the 2013/14 normal seeding plots and the 2014 late seeding datasets, respectively. Three lines (line 2, 88 and 91) were in the top yield groups under both environments (Table 4.11), and could be regarded as lines with good heat tolerance. When comparing the mean of each of the flowering and yield component traits (DTF, DTFT, DOF, Pod, Seed and SSW) between the best yield group and worst yield group at normal seeding, only differences in flowering traits were significant. The top yielders appeared to flower late but have a longer flowering duration. The lack of large differences in yield component traits on the main-stem between normal and late seeding were in agreement with the modest correlation results in this study. However, for the main-stem, pod number was the most important component associated with driving main-stem yield. Overall, no single yield component demonstrated a significant association with final grain yield (Table 4.11). Although at late seeding the difference in each of the traits between the top and bottom groups were generally not significant (no difference in any of these traits between top and bottom groups

exceed each the LSD value for the corresponding trait), still the high yield group tended to have characteristics of late start of flowering and a longer flowering duration.

6.0 CONCLUSION AND FUTURE RESEARCH

Analysis of variance for the measured traits demonstrated that genotypes differed significantly in the RIL population derived from the cross of CDC Centennial X CDC Sage, which supported my hypothesis that the RIL population PR-11 could produce a wide genetic variation in flowering and yield related traits. Further, through SNP markers, the derived linkage map had stable QTLs for all nine traits except Rnode, which were identified across normal seeding environments. Some different QTLs were detected at the late seeded environment, but more years experiments are needed to verify the stability and precision of these QTLs.

Correlation and path coefficient analysis revealed that long flowering duration and high pod number per plant were promising and helpful indices for high yield potential under both normal and heat stress environments. In Canada, a short period of extreme high temperature is the most pervasive type of heat stress. It can cause the abscission of reproductive organs in pea, and cultivars with a long flowering time could take advantage of indeterminate growth habit and flower again (in most cases, a longer flowering time means the development of more branches and the setting of more pods on the branches, thereby increasing the total pod number per plant and final yield). Three lines (PR-11-2, PR-11-88, PR-11-91) out of the eight highest yielding lines were consistently top performers and selected at both normal and late seeding dates, and they can be considered as the best heat tolerant lines from PR-11.

The difficulty of setting up a reliable field environment for the screen of heat resistance poses a challenge. Although late seeding in this study brought more heat stress to the population, the heat stress was still insufficient to cause significant damage in most of the yield component traits. In future research, the population should be sown at more and different dates. Besides, a thorough screen of heat resistance traits such as canopy temperature depression, stay-green

ability and flowering and yield related traits in well-known pea cultivars (as reference checks) is also needed, because the parents of PR-11 (CDC Centennial and CDC Sage) failed to display a large variation in just the flowering traits measured.

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APPENDICES

Appendix A. Product name of herbicides with scientific names and foumulation.

Product name	Common name	Formulation
Axial	Pinoxaden	50g/L EC
Basagran	Bentazon	480g/L SN
Centurion	Clethodim	240g/L EC
Edge	Ethalfluralin	5% G
Pursuit	Imazethapyr	240g/L SN
Reglone	Diquat	240g/L SN
Roundup	Glyphosate K + salt	540g/L SN
Viper	Imazamox: bentazon	20g/L: 429g/L SN

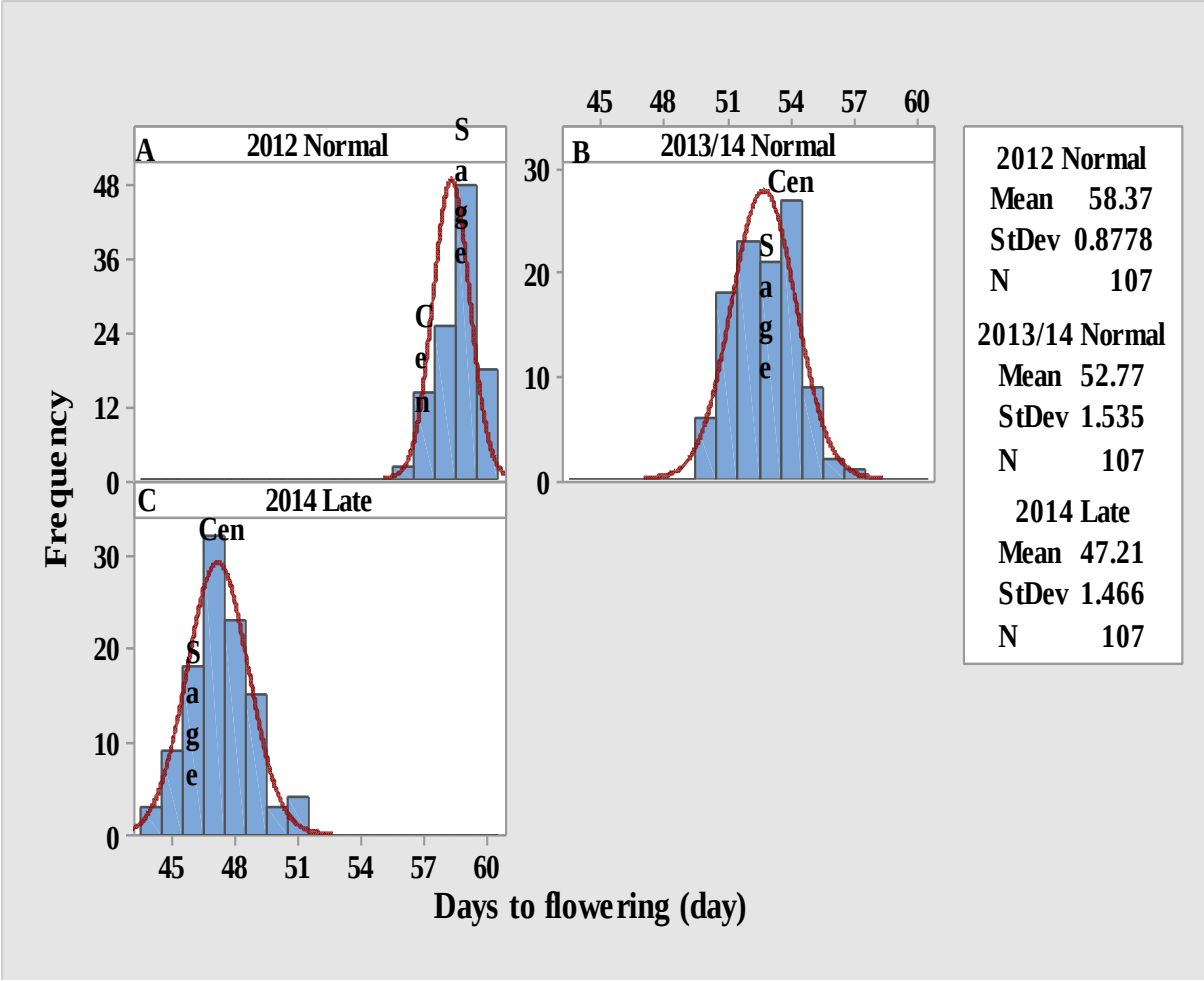
EC: emulsifiable concentrate; G: granule; SN: solution.
For more details, refer to the "2015 Guide to Crop Protection" by Saskatchewan Ministry of Agriculture.

Appendix B. Mean, standard deviation (SD), minimum and maximum of nine traits for 107 RILs of the CDC Centennial X CDC Sage population, and the means of the parental cultivars based on the 2012 Normal seeding date dataset.

CDC Centennial X CDC Sage population					Parental cultivars	
					Mean	
Variable	Mean	Std Dev	Minimum	Maximum	Centennial	Sage
DTF	58.4	0.88	55.5	60.0	57.0	59.0
DTFT	79.9	3.08	74.0	85.0	84.1	80.0
DOF	21.5	3.04	15.0	28.0	27.0	21.0
Node	20.2	1.05	17.6	22.7	20.6	20.7
Rnode	7.6	1.30	4.8	11.0	8.0	8.0
Pod	5.9	1.27	3.5	10.0	6.5	5.5
Yield	464.7	70.52	290.4	615.4	550.5	397.3

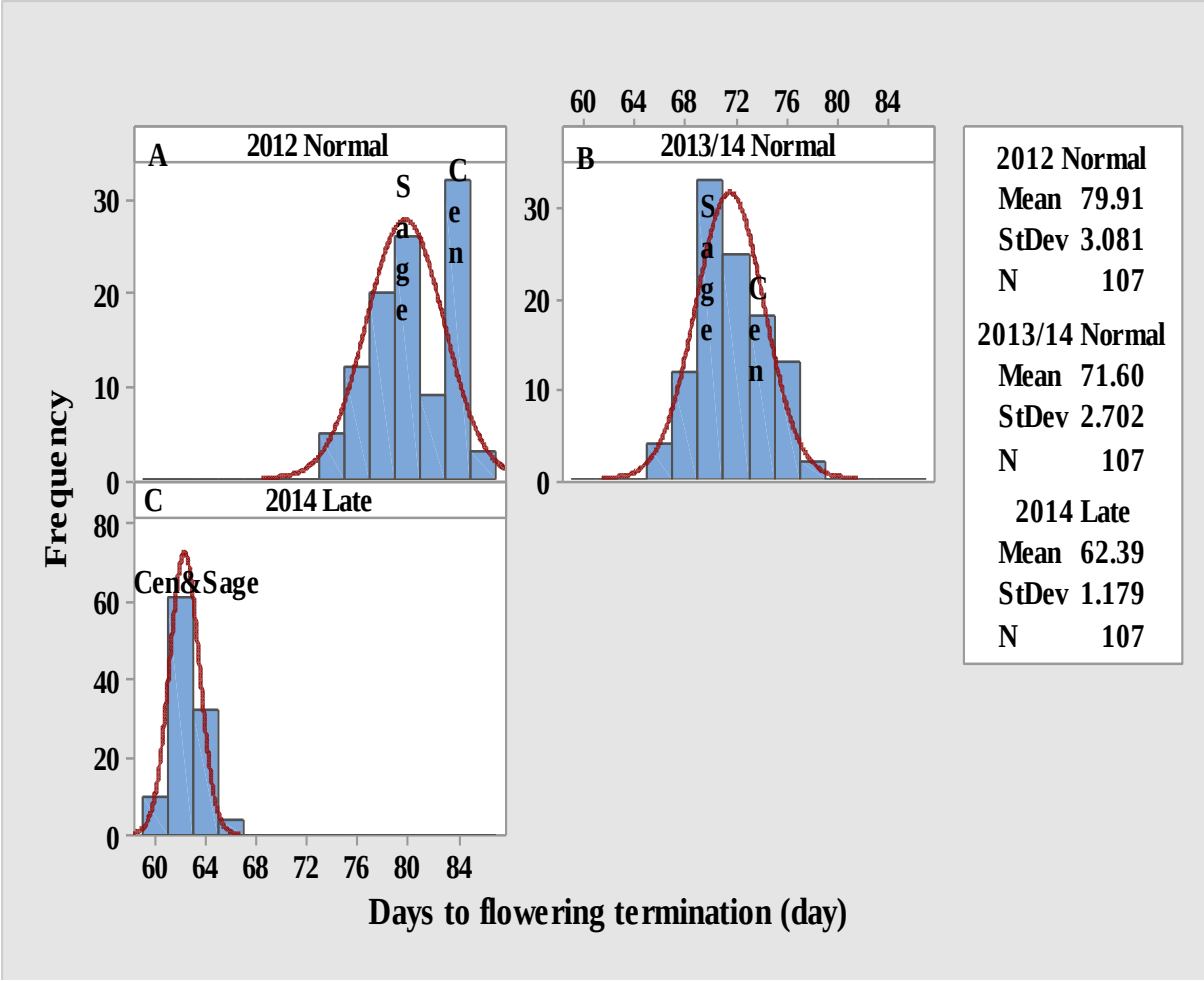
Notes: For explanation of DTF, DTFT, DOF, Node, Rnode, Pod and Yield, refer to Table 4.4. Seed and SSW were not measured in 2012.

Appendix C. Frequency distribution of days to flowering (DTF) for 107 RILs of the CDC Centennial X CDC Sage population.



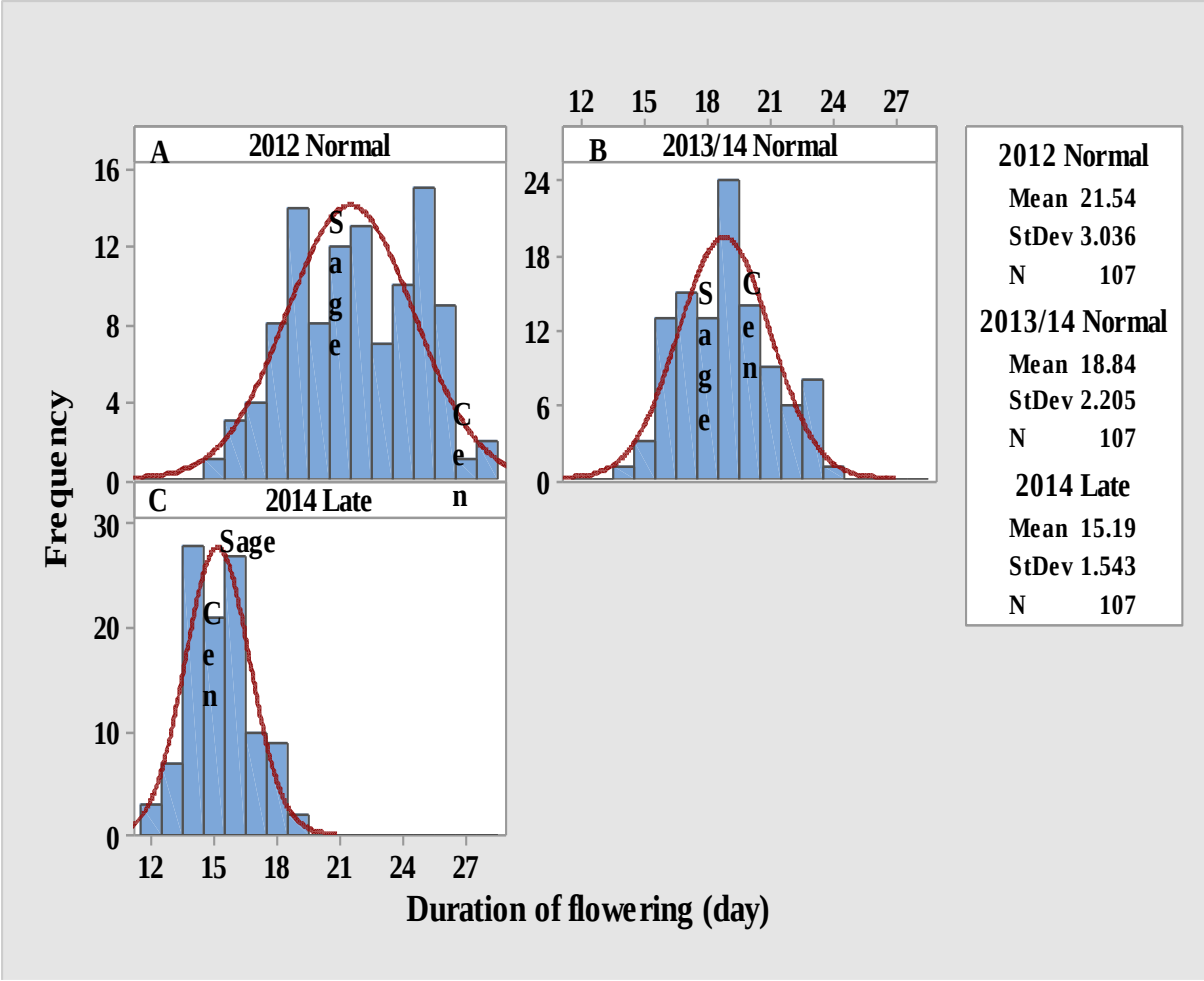
Panel A is based on RILs from the dataset 2012 Normal seeding date, where 107 genotypes were averaged over two blocks. Panel B is based on RILs from the dataset 2013/14 Normal, where 107 genotypes were averaged over two blocks at each of the three locations. Panel C is based on RILs from the dataset 2014 Late seeding date, where 107 genotypes were averaged over two blocks. Cen: CDC Centennial; Sage: CDC Sage.

Appendix D. Frequency distribution of days to flowering termination (DTFT) for 107 RILs of the CDC Centennial X CDC Sage population.



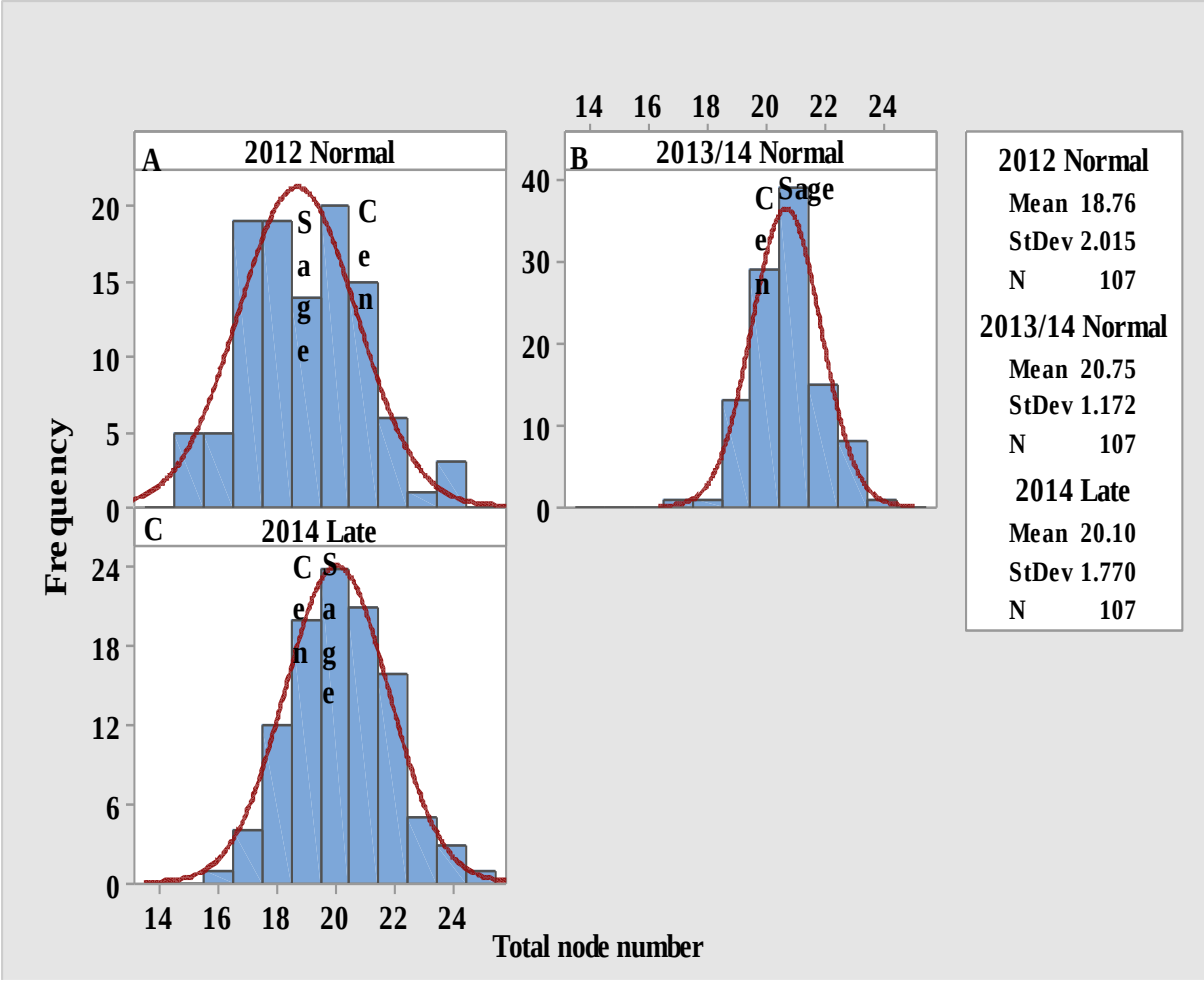
Panel A is based on RILs from the dataset 2012 Normal seeding date, where 107 genotypes were averaged over two blocks. Panel B is based on RILs from the dataset 2013/14 Normal, where 107 genotypes were averaged over two blocks at each of the three locations. Panel C is based on RILs from the dataset 2014 Late seeding date, where 107 genotypes were averaged over two blocks. Cen: CDC Centennial; Sage: CDC Sage.

Appendix E. Frequency distribution of duration of flowering (DOF) for 107 RILs of the CDC Centennial X CDC Sage population.



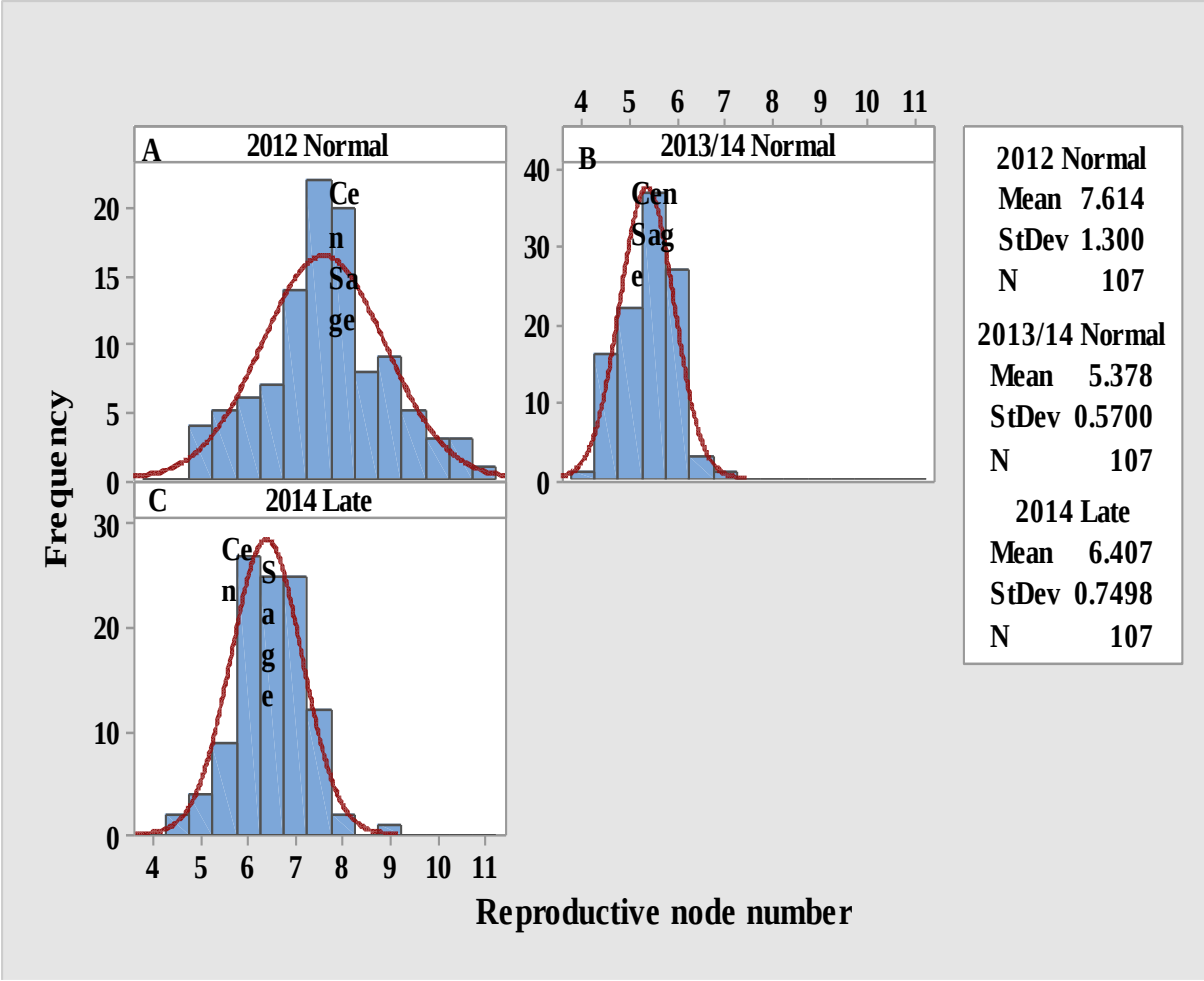
Panel A is based on RILs from the dataset 2012 Normal seeding date, where 107 genotypes were averaged over two blocks. Panel B is based on RILs from the dataset 2013/14 Normal, where 107 genotypes were averaged over two blocks at each of the three locations. Panel C is based on RILs from the dataset 2014 Late seeding date, where 107 genotypes were averaged over two blocks. Cen: CDC Centennial; Sage: CDC Sage.

Appendix F. Frequency distribution of total node number for 107 RILs of the CDC Centennial X CDC Sage population.



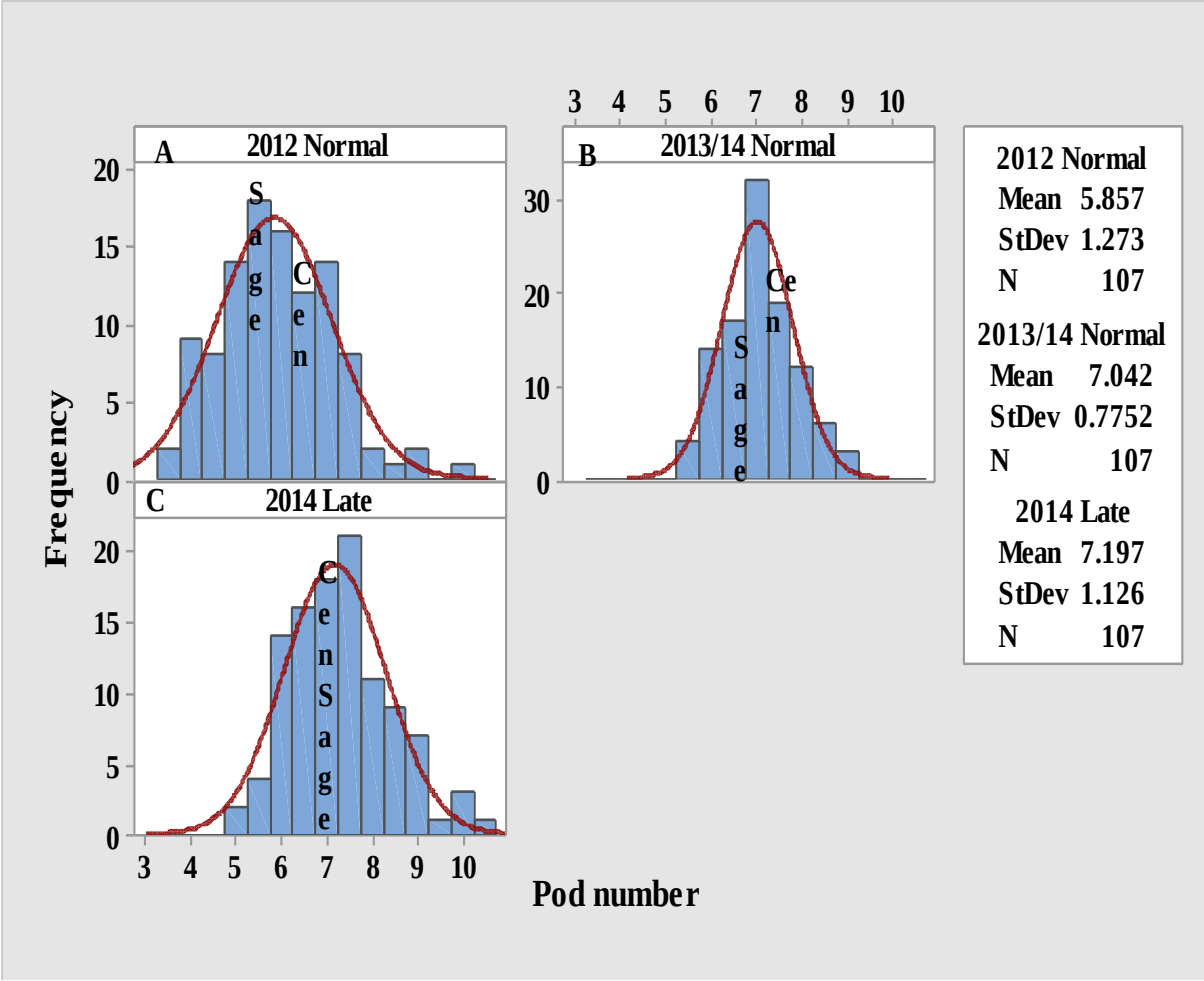
Panel A is based on RILs from the dataset 2012 Normal seeding date, where 107 genotypes were averaged over two blocks. Panel B is based on RILs from the dataset 2013/14 Normal, where 107 genotypes were averaged over two blocks at each of the three locations. Panel C is based on RILs from the dataset 2014 Late seeding date, where 107 genotypes were averaged over two blocks. Cen: CDC Centennial; Sage: CDC Sage.

Appendix G. Frequency distribution of reproductive node number for 107 RILs of the CDC Centennial X CDC Sage population.



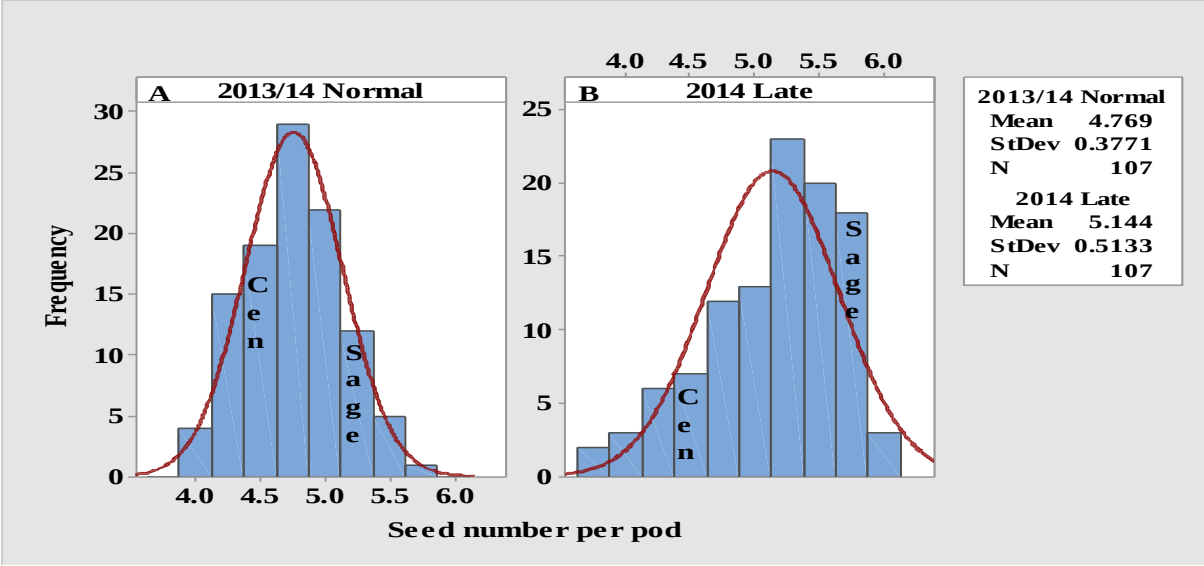
Panel A is based on RILs from the dataset 2012 Normal seeding date, where 107 genotypes were averaged over two blocks. Panel B is based on RILs from the dataset 2013/14 Normal, where 107 genotypes were averaged over two blocks at each of the three locations. Panel C is based on RILs from the dataset 2014 Late seeding date, where 107 genotypes were averaged over two blocks. Cen: CDC Centennial; Sage: CDC Sage.

Appendix H. Frequency distribution of pod number for 107 RILs of the CDC Centennial X CDC Sage population.



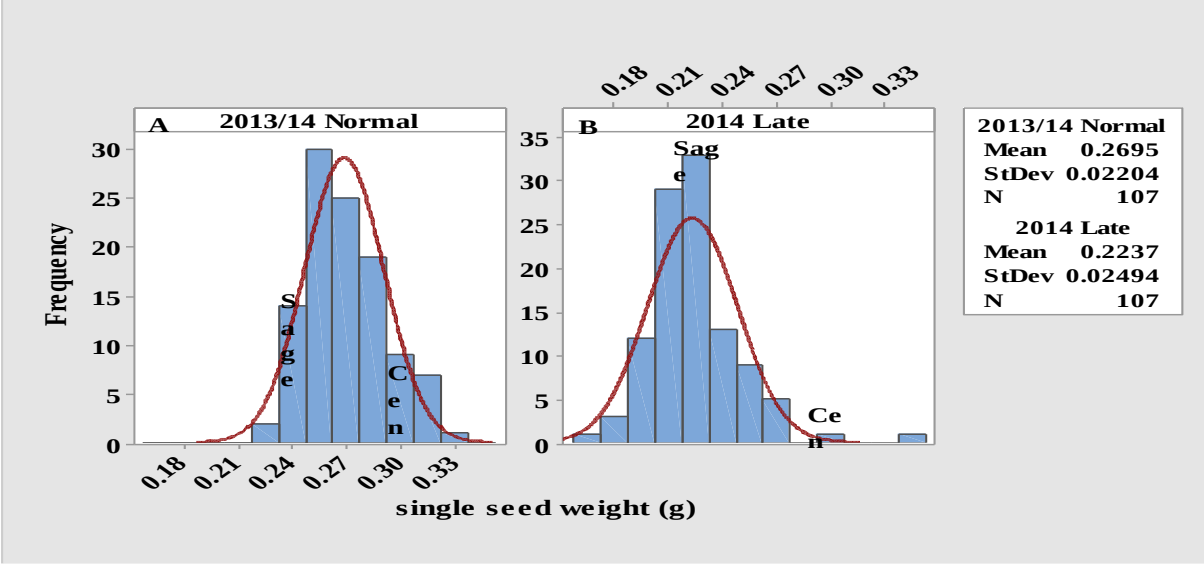
Panel A is based on RILs from the dataset 2012 Normal seeding date, where 107 genotypes were averaged over two blocks. Panel B is based on RILs from the dataset 2013/14 Normal, where 107 genotypes were averaged over two blocks at each of the three locations. Panel C is based on RILs from the dataset 2014 Late seeding date, where 107 genotypes were averaged over two blocks. Cen: CDC Centennial; Sage: CDC Sage.

Appendix I. Frequency distribution of seed number per pod for 107 RILs of the CDC Centennial X CDC Sage population.



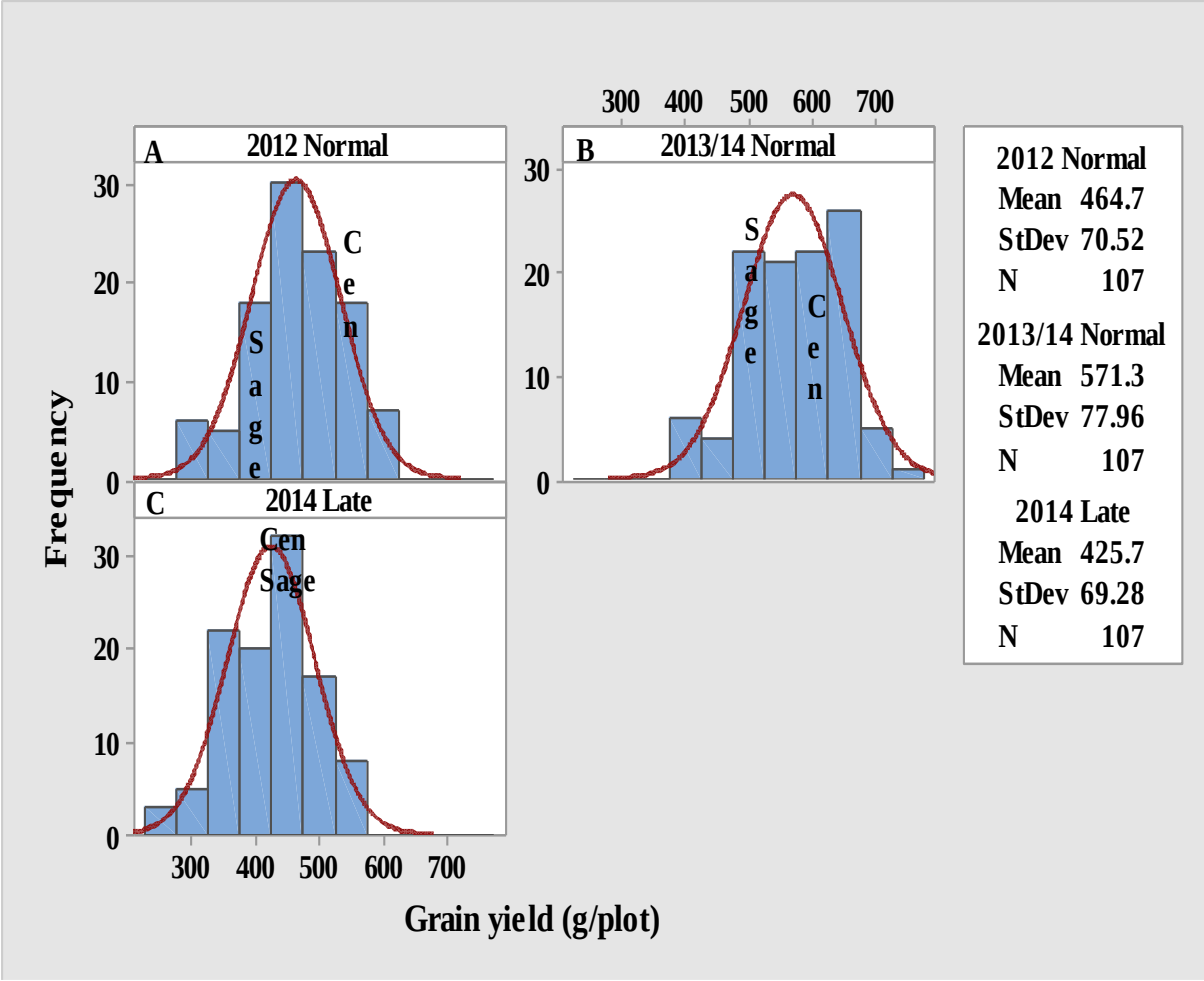
Panel A is based on RILs from the dataset 2012 Normal seeding date, where 107 genotypes were averaged over two blocks. Panel B is based on RILs from the dataset 2013/14 Normal, where 107 genotypes were averaged over two blocks at each of the three locations. Panel C is based on RILs from the dataset 2014 Late seeding date, where 107 genotypes were averaged over two blocks. Cen: CDC Centennial; Sage: CDC Sage.

Appendix J. Frequency distribution of single seed weight for 107 RILs of the CDC Centennial X CDC Sage populations.



Panel A is based on RILs from the dataset 2012 Normal seeding date, where 107 genotypes were averaged over two blocks. Panel B is based on RILs from the dataset 2013/14 Normal, where 107 genotypes were averaged over two blocks at each of the three locations. Panel C is based on RILs from the dataset 2014 Late seeding date, where 107 genotypes were averaged over two blocks. Cen: CDC Centennial; Sage: CDC Sage.

Appendix K. Frequency distribution of grain yield for 107 RILs of the CDC Centennial X CDC Sage population.



Panel A is based on RILs from the dataset 2012 Normal seeding date, where 107 genotypes were averaged over two blocks. Panel B is based on RILs from the dataset 2013/14 Normal, where 107 genotypes were averaged over two blocks at each of the three locations. Panel C is based on RILs from the dataset 2014 Late seeding date, where 107 genotypes were averaged over two blocks. Cen: CDC Centennial; Sage: CDC Sage.

Appendix L. Correlations between DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW and plot yield in PR-11 based on the 2012 Normal seeding date dataset.

	DTF	DTFT	DOF	Node	Rnode	Pod	Yield
DTF	-	0.19*	-0.09	0.07	-0.17	-0.11	0.06
DTFT		-	0.96***	0.09	0.21*	0.30**	-0.09
DOF			-	0.07	0.27**	0.34***	-0.10
Node				-	0.26**	0.14	0.23*
Rnode					-	0.67***	-0.09
Pod						-	-0.06
Yield							-

Notes: For the explanations of DTF, DTFT, DOF, Node, Rnode, Pod, Seed, SSW and yield, refer to Table 4.4.
NS: not significant; *: significant at l $P \leq 0.05$; **: significant at $P \leq 0.01$; ***: significant at $P \leq 0.001$.

Appendix M. Estimates of partial variance components and heritability calculations for DTF, DOF, Node and yield for 107 RILs of CDC Centennial X CDC Sage grown at Rosthern in 2012 and 2013.

Variance Components	DTF	DOF	Node	Yield
σ^2_g	0.13 ±0.18	0.23 ±1.24	0.27 ±0.32	3228.5 ±1248.6
σ^2_{gl}	0.85 ±0.25	3.39 ±1.93	0.37 ±0.48	1546.8 ±1466.8
σ^2_e	1.76 ±0.17	18.28 ±1.77	5.14 ±0.50	15336 ±1482.6
σ^2_p	1.00	6.50	1.74	7835.9
h^2	0.13	0.04	0.16	0.41

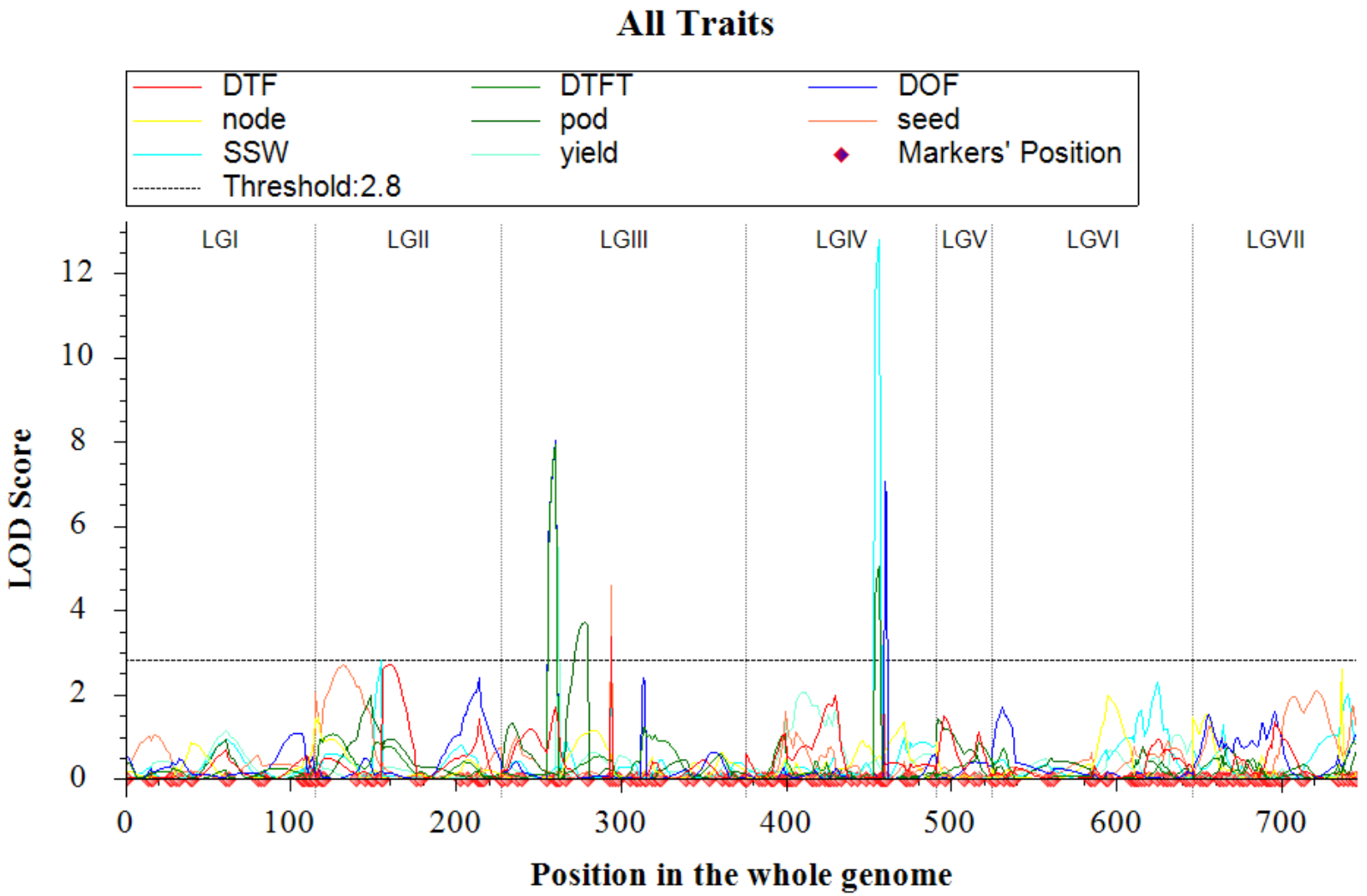
For the explanations of DTF, DOF, Node and Yield, refer to Table 4.4.
The heritability of the traits days to flowering termination, reproductive node number on the main-stem, pod numbers on the main-stem, seed numbers per pod and single seed weight are not included, as the σ^2_g of these traits showed negative values.

Appendix N. Eight highest and lowest yield lines of the RILs population from the 2013/14 Normal seeding and 2014 Late seeding environments for DTF, DTFT, DOF, Pod, Seed and SSW.

2013/14 Normal							2014 Late						
Parental lines	DTF	DTFT	DOF	Pod	Seed	SSW	Parental lines	DTF	DTFT	DOF	Pod	Seed	SSW
CDC Centennial	54.1	74.1	20.0	7.5	4.4	0.3027	CDC Centennial	47.3	62.5	15.0	6.9	4.6	0.2401
CDC Sage	52.6	70.6	17.9	6.7	5.4	0.2317	CDC Sage	46.4	62.0	16.0	6.9	5.7	0.1976
High yield lines	DTF	DTFT	DOF	Pod	Seed	SSW	High yield lines	DTF	DTFT	DOF	Pod	Seed	SSW
PR-11-2	53.8	74.3	20.6	7.8	4.2	0.2830	PR-11-2	48.2	66.3	17.8	9.1	4.8	0.2229
PR-11-88	54.1	70.3	16.3	6.4	5.1	0.2628	PR-11-88	49.0	64.0	15.5	7.9	5.3	0.2100
PR-11-91	54.2	75.2	21.0	6.8	4.2	0.2682	PR-11-91	49.0	65.0	16.0	8.3	3.7	0.2247
PR-11-54	52.0	74.8	22.8	8.2	4.8	0.2494	PR-11-7	51.0	64.3	13.3	7.3	5.9	0.2099
PR-11-67	50.6	73.2	22.6	7.1	4.6	0.2718	PR-11-15	48.3	63.5	15.3	5.3	4.5	0.1627
PR-11-70	57.0	75.8	18.8	5.7	4.3	0.3139	PR-11-18	45.8	63.0	17.3	7.3	5.5	0.2119
PR-11-83	52.5	71.9	19.4	7.1	4.8	0.2835	PR-11-29	48.0	62.0	14.0	6.4	4.6	0.2575
PR-11-98	55.1	77.6	22.5	6.5	5.0	0.2468	PR-11-44	46.3	63.5	17.3	6.4	5.1	0.3438
Mean	53.6	74.1	20.5	7.0	4.6	0.2724	Mean	48.2	63.9	15.8	7.2	4.9	0.2304
Low yield lines	DTF	DTFT	DOF	Pod	Seed	SSW	Low yield lines	DTF	DTFT	DOF	Pod	Seed	SSW
PR-11-20	51.1	69.6	18.5	5.6	4.8	0.2675	PR-11-20	45.8	61.3	15.5	6.0	6.0	0.2349
PR-11-38	49.7	66.7	17.0	6.1	4.2	0.2982	PR-11-38	46.5	60.8	14.3	6.8	4.6	0.2704
PR-11-64	51.8	67.0	15.2	7.4	4.5	0.2359	PR-11-64	47.3	63.3	16.0	7.3	5.7	0.2552
PR-11-73	50.5	66.2	15.7	5.8	4.3	0.3109	PR-11-73	45.3	60.5	15.3	6.1	5.0	0.2670
PR-11-90	53.0	70.9	17.9	6.3	5.1	0.2384	PR-11-90	47.3	62.8	15.5	6.1	6.0	0.1792
PR-11-23	50.3	67.5	17.2	6.8	4.8	0.2913	PR-11-27	46.8	60.8	14.0	6.0	5.7	0.2117
PR-11-31	50.0	67.1	17.1	8.5	4.1	0.2840	PR-11-58	48.3	61.5	13.3	6.8	5.7	0.2012
PR-11-106	52.1	67.9	15.8	8.4	4.9	0.2314	PR-11-80	47.0	62.5	15.5	6.5	5.2	0.2204
Mean	51.1	67.9	16.8	6.8	4.6	0.2698	Mean	46.8	61.7	14.9	6.4	5.5	0.2300
LSD	3.1	5.7	4.7	1.7	0.8	0.030	LSD	2.1	2.7	3.1	2.6	1.2	0.060

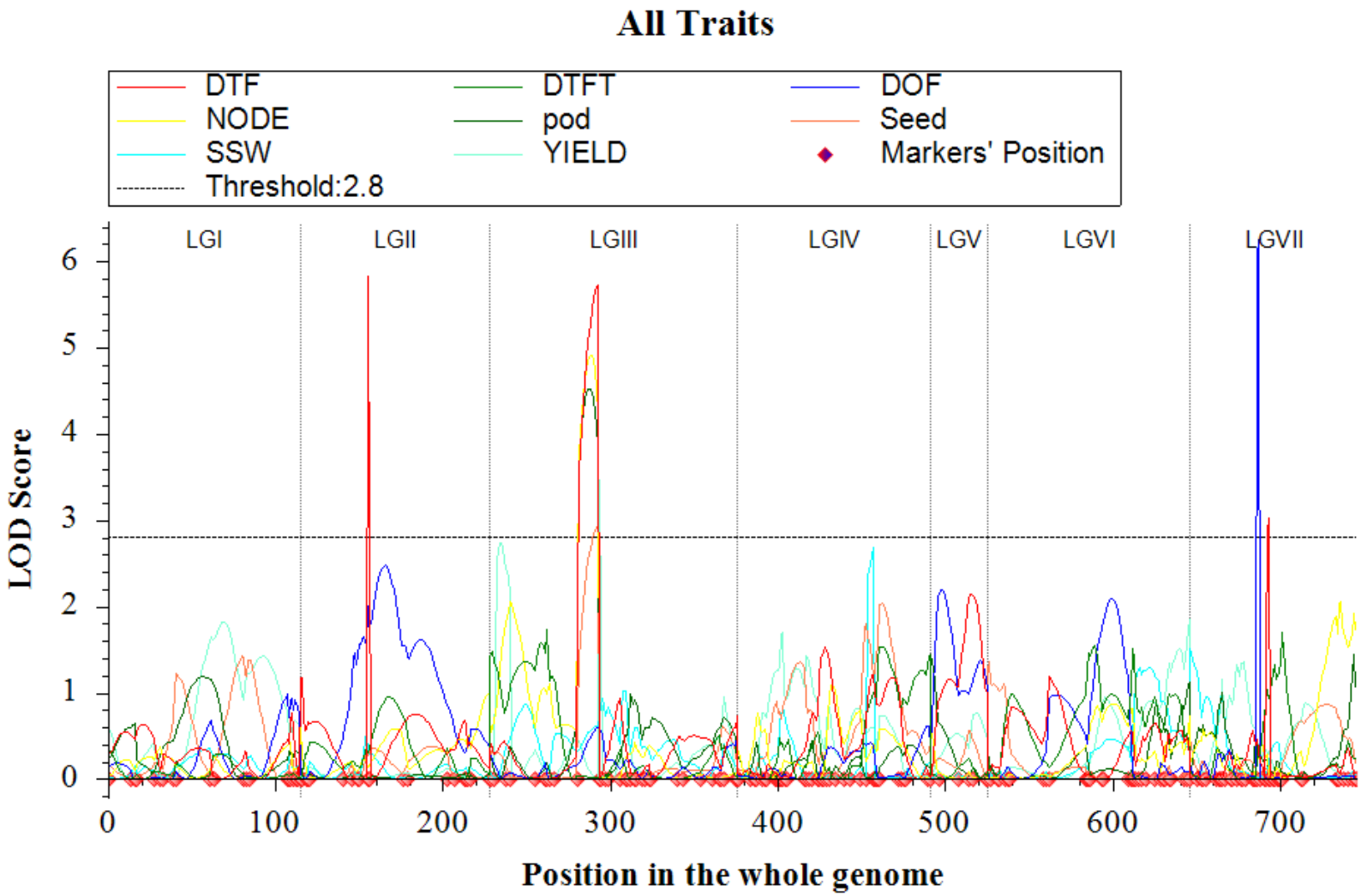
For the explanation of DTF, DTFT, DOF, Pod, Seed and SSW, refer to Table 4.4.

Appendix O. LOD profile for DTF, DTFT, DOF, Node, Pod, Seed, SSW and yield under 2013 and 2014 normal seeding dates.



Notes: For the explanation of DTF, DTFT, DOF, Node, Pod, Seed, SSW and yield, refer to Table 4.4.

Appendix P. LOD profile for DTF, DTFT, DOF, Node, Pod, Seed, SSW and yield at 2014 late seeding date.



Notes: For the explanation of DTF, DTFT, DOF, Node, Pod, Seed, SSW and yield, refer to Table 4.4.